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(54) NOUVEAUX PROCEDES DE SELECTION

(54) INVERSION OF THE NEGATIVE-SELECTIVE EFFECT OF NEGATIVE MARKER PROTEINS USING SELECTION METHODS

(57)

The invention relates to methods for producing transformed plant cells or organisms by transforming a population of plant cells comprising at least one marker protein having a directly or indirectly toxic effect therefor, by means of at least one nucleic acid sequence to be inserted, said sequence being combined with at least one compound preferably a DNA construct which is able to reduce the expression, quantity, activity and/or function of the marker protein. The transformed plant cells have a growth advantage in relation to the non-transformed cells as a result of the action of said compound.

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(54) Title: INVERSION OF THE NEGATIVE-SELECTIVE EFFECT OF NEGATIVE MARKER PROTEINS USING SELECTION METHODS

(57) Abrégé/Abstract:
The invention relates to methods for producing transformed plant cells or organisms by transforming a population of plant cells comprising at least one marker protein having a directly or indirectly toxic effect therefor, by means of at least one nucleic acid sequence to be inserted, said sequence being combined with at least one compound preferably a DNA construct which is able to reduce the expression, quantity, activity and/or function of the marker protein. The transformed plant cells have a growth advantage in relation to the non-transformed cells as a result of the action of said compound.

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(54) Title: INVERSION OF THE NEGATIVE-SELECTIVE EFFECT OF NEGATIVE MARKER PROTEINS USING SELECTION METHODS

(54) Bezeichnung: REVERTIERUNG DER NEGATIV-SELEKTIVEN WIRKUNG VON NEGATIVEN MARKERPROTEINEN ALS SELEKTIONSVERFAHREN

(57) Abstract: The invention relates to methods for producing transformed plant cells or organisms by transforming a population of plant cells comprising at least one marker protein having a directly or indirectly toxic effect therefor, by means of at least one nucleic acid sequence to be inserted, said sequence being combined with at least one compound preferably a DNA construct which is able to reduce the expression, quantity, activity and/or function of the marker protein. The transformed plant cells have a growth advantage in relation to the non-transformed cells as a result of the action of said compound.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft Verfahren zur Herstellung transformierter pflanzlicher Zellen oder Organismen durch Transformation einer Population pflanzlicher Zellen, die mindestens ein Markerprotein mit einem für diese direkt oder indirekt toxischen Effekt umfasst, mit mindestens einer zu insertierenden Nukleinsäuresequenz in Kombination mit mindestens einer Verbindung - bevorzugt einem DNA-Konstrukt - befähigt zur Verminderung der Expression, Menge, Aktivität und/oder Funktion des Markerproteins, wobei die transformierten pflanzlichen Zellen infolge der Wirkung besagter Verbindung gegenüber nicht-transformierten Zellen einen Wachstumsvorteil haben.

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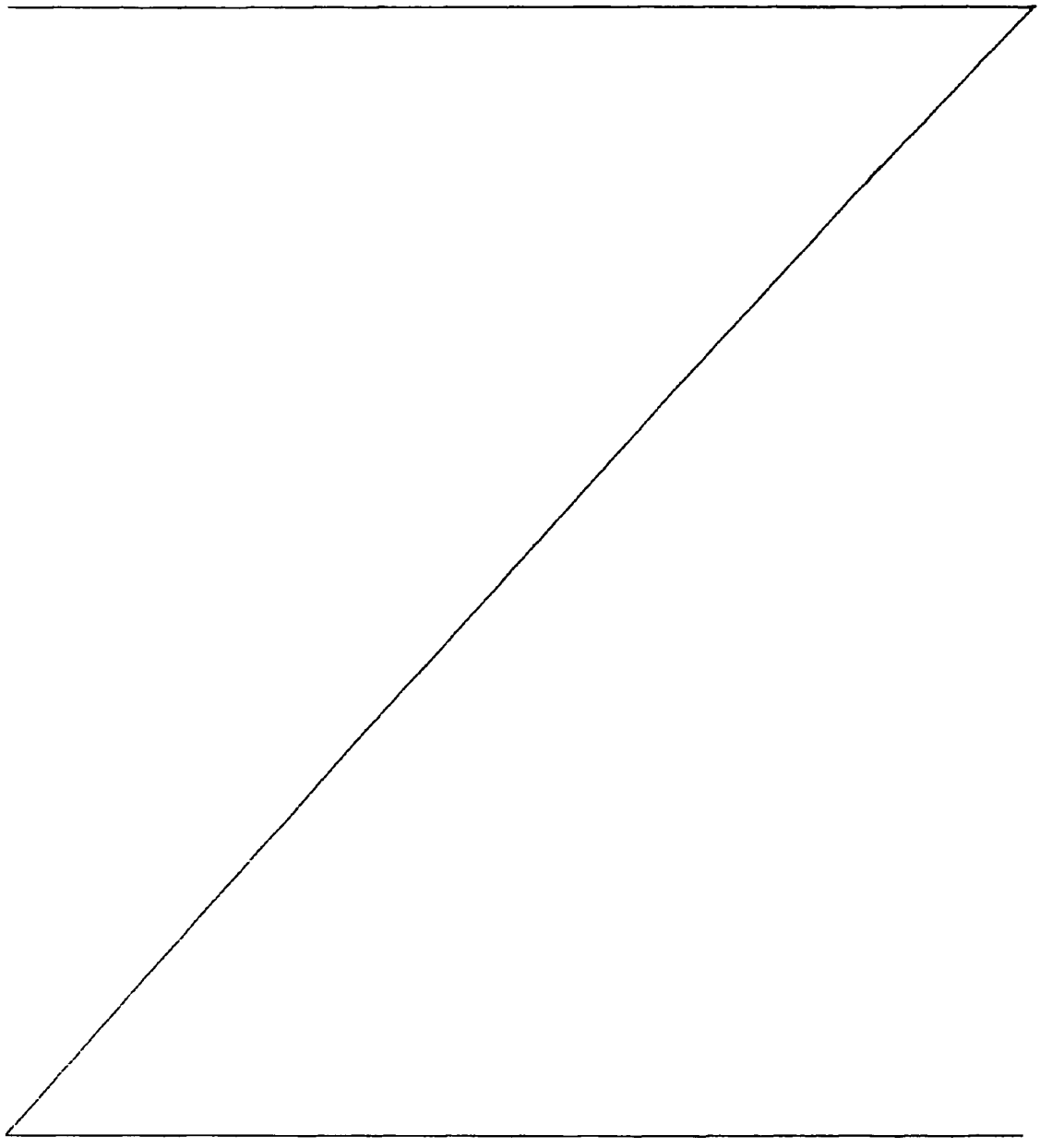
INVERSION OF THE NEGATIVE-SELECTIVE EFFECT OF NEGATIVE MARKER PROTEINS USING SELECTION METHODS

Description

The present invention relates to processes for preparing transformed plant cells or organisms by transforming a population of plant cells which comprises at least one marker protein having a direct or indirect toxic effect for said population, with at least one nucleic acid sequence to be inserted in combination with at least one compound, preferably a DNA construct, capable of reducing the expression, amount, activity and/or function of the marker protein, with the transformed plant cells having a growth advantage over nontransformed cells, due to the action of said compound.

Genetic material is successfully introduced usually only into a very limited number of target cells of a population. This necessitates the distinction and isolation of successfully transformed from nontransformed cells, a process which is referred to as selection. Traditionally, the selection is carried out by way of a "positive" selection, wherein the transformed cell is enabled to grow and to survive, whereas the untransformed cell is inhibited in its growth or destroyed (McCormick et al. (1986) Plant Cell Reports 5:81-84). A positive selection of this kind is usually implemented by genes which code for a resistance to a biocide (e.g. a herbicide such as phosphinothricin, glyphosate or bromoxynil, a metabolism inhibitor such as 2-deoxyglucose 6-phosphate (WO 98/45456) or an antibiotic such as tetracycline, ampicillin, kanamycin, G 418, neomycin, bleomycin or hygromycin). Such genes are also referred to as positive selection markers. The positive selection marker is coupled (physically or by means of cotransformation) to the nucleic acid sequence to be introduced into the cell genome and is then introduced into the cell. Subsequently, the cells are cultured on a medium under the appropriate selection pressure (for example in the presence of an appropriate antibiotic or herbicide), whereby the transformed cells, owing to the required resistance to said selection pressure, have a growth/survival advantage and can thus be selected. Positive selection markers which may be mentioned by way of example are:

- phosphinothricin acetyltransferases (PAT) (also: Bialophos® resistance; bar) acetylate the free amino group of the glutamine synthase inhibitor phosphinothricin (PPT) and thus achieve a detoxification (de Block et al. (1987) EMBO J



6:2513-2518; Vickers JE et al. (1996) Plant Mol Biol Reporter 14:363–368; Thompson CJ et al. (1987) EMBO J 6:2519–2523).

- 5-enolpyruvylshikimate 3-phosphate synthases (EPSPS) impart a resistance to the unselective herbicide Glyphosat® (N-(phosphonomethyl)glycine; Steinrucken HC et al. (1980) Biochem Biophys Res Commun 94:1207–1212; Levin JG and Sprinson DB (1964) J Biol Chem 239:1142–1150; Cole DJ (1985) Mode of action of glyphosate; A literature analysis, p. 48–74. In: Grossbard E and Atkinson D (eds.) The herbicide glyphosate. Buttersworths, Boston.). Glyphosate-tolerant EPSPS variants for use as selection markers have been described (Padgette SR et al. (1996). New weed control opportunities: development of soybeans with a Roundup Ready™ gene. In: Herbicide Resistant Crops (Duke SO, ed.), pp. 53-84. CRC Press, Boca Raton, FL; Saroha MK and Malik VS (1998) J Plant Biochemistry and Biotechnology 7:65–72; Padgette SR et al.(1995) Crop Science 35(5):1451–1461; US 5,510,471; US 5,776,760; US 5,864,425; US 5,633,435; US 5,627,061; US 5,463,175; EP-A 0 218 571).

- neomycin phosphotransferases constantly impart a resistance to aminoglycoside antibiotics such as neomycin, G418, hygromycin, paromomycin or kanamycin by reducing the inhibiting action thereof by means of a phosphorylation reaction (Beck et al. (1982) Gene 19:327-336).

- 2-deoxyglucose 6-phosphate phosphatases impart a resistance to 2-deoxyglucose (EP-A 0 807 836; Randez-Gil et al. (1995) Yeast 11:1233-1240; Sanz et al. (1994) Yeast 10:1195-1202).

- acetolactate synthases impart a resistance to imidazolinone/sulfonylurea herbicides (e.g. imazzamox, imazapyr, imazaquin, imazethapyr, amidosulforon, azimsulfuron, chlorimuron ethyl, chlorsulfuron; Sathasivan K et al. (1990) Nucleic Acids Res 18(8):2188).

In addition, resistance genes to the antibiotics hygromycin (hygromycin phosphotransferases), chloramphenicol (chloramphenicol acetyltransferase), tetracycline, streptomycin, zeocine and ampicillin (ß-lactamase gene; Datta N, Richmond MH.(1966) Biochem J 98(1):204-9) have been described.

Genes such as isopentenyl transferase (ipt) from Agrobacterium tumefaciens (strain:PO22) (GenBank Acc. No.: AB025109) may likewise be used as selection markers. The ipt gene is a key enzyme of cytokine biosynthesis. Its overexpression facilitates the re-

generation of plants (e.g. selection on cytokine-free medium) (Ebinuma H et al. (2000) Proc Natl Acad Sci USA 94:2117-2121; Ebinuma H et al. (2000) Selection of Marker-free transgenic plants using the oncogenes (*ipt*, *rol* A, B, C) of Agrobacterium as selectable markers, In Molecular Biology of Woody Plants. Kluwer Academic Publishers). The disadvantages here are, firstly, the fact that the selection disadvantage is based on usually subtle differences in cell proliferation and, secondly, the fact that the plant acquires unwanted properties (gall tumor formation) due to transformation with an oncogene.

EP-A 0 601 092 describes various other positive selection markers. Examples which may be mentioned are: β-glucuronidase (in connection with, for example, cytokinine glucuronide), mannose 6-phosphate isomerase (in connection with mannose), UDP-galactose 4-epimerase (in connection with galactose, for example).

Negative selection markers are used for selecting organisms in which marker sequences have been successfully deleted (Koprek T et al. (1999) Plant J 19(6):719-726). In the presence of a negative selection marker, the corresponding cell is destroyed or experiences a growth disadvantage. Negative selection involves, for example, the negative selection marker introduced into the plant converting a compound which otherwise has no action disadvantageous to the plant into a compound with a disadvantageous (i.e. toxic) action. Examples of negative selection markers include: thymidine kinase (TK), for example of Herpes simplex virus (Wigler et al. (1977) Cell 11:223), cellular adenine phosphoribosyl transferase (APRT) (Wigler et al. (1979) Proc Natl Acad Sci USA 76:1373), hypoxanthine phosphoribosyl transferase (HPRT) (Jolly et al. (1983) Proc Natl Acad Sci USA 80:477), diphtheria toxin A fragment (DT-A), the bacterial xanthine-guanine phosphoribosyl transferase (gpt; Besnard et al. (1987) Mol. Cell. Biol. 7:4139; Mzoz and Moolten (1993) Human Gene Therapy 4:589-595), the codA gene product coding for a cytosine deaminase (Gleave AP et al. (1999) Plant Mol Biol. 40(2):223-35; Perera RJ et al. (1993) Plant Mol Biol 23(4): 793-799; Stougaard J; (1993) Plant J 3:755-761; EP-A1 595 873), the cytochrome P450 gene (Koprek et al. (1999) Plant J 16:719-726), genes coding for a haloalkane dehalogenase (Naested H (1999) Plant J 18:571-576), the iaaH gene (Sundaresan V et al. (1995) Genes & Development 9:1797-1810) or the tms2 gene (Fedoroff NV & Smith DL (1993) Plant J 3: 273-289). The negative selection markers are usually employed in combination with "prodrugs" or "pro-toxins", compounds which are converted into toxins by the activity of the selection marker.

5-Methylthioribose (MTR) kinase is an enzyme whose enzymic activity in plants, bacteria and protozoa, but not in mammals, has been described. The enzyme may convert an MTR analog (5-(triromethyl)thioribose) as a "subversive substrate" of the methionine salvage pathway via an unstable intermediate to give the toxic compound carbothionyl difluoride.

Said selection systems have various disadvantages. The introduced selection marker (e.g. resistance to antibiotics) is justified only during transformation and selection but is later a usually unnecessary and often also undesired protein product. This may be disadvantageous for reasons of consumer acceptance and/or approval as a food and/or feed product. Another disadvantage in this connection is the fact that the selection marker used for selection is usually genetically coupled to the nucleic acid sequence to be inserted into the genome and cannot be decoupled by segregation during propagation or crossing. Usually, deletion of the marker sequence is required, making additional steps necessary. In addition, biotechnological studies require in numerous cases multiple transformation with various gene constructs. Here, each transformation step requires a new selection marker unless the previously used marker is to be laboriously deleted first. This, however, necessitates a broad palette of well-functioning selection markers which are not available for most plant organisms.

Consequently, it was the object of the invention to provide novel selection processes for selecting transformed plant cells and organisms, which, if possible, no longer have the disadvantages of the available systems. This object is achieved by the present invention.

The invention firstly relates to a process for preparing transformed plant cells or organisms, which process comprises the following steps:

a) transforming a population of plant cells, with the cells of said population containing at least one marker protein capable of causing directly or indirectly a toxic effect for said population, with at least one nucleic acid sequence to be inserted in combination with at least one compound capable of reducing the expression, amount, activity and/or function of at least one marker protein, and

b) selecting transformed plant cells whose genome contains said nucleic acid sequence and which have a growth advan-

tage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells.

In a preferred embodiment, the marker protein is a protein capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population. In this case, the process of the invention preferably comprises the following steps:

a) transforming the population of plant cells with at least one nucleic acid sequence to be inserted in combination with at least one compound capable of reducing the expression, amount, activity and/or function of at least one marker protein, and

b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and

c) selecting transformed plant cells whose genome contains said inserted nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells.

The nontoxic substance X is preferably a substance which does not naturally occur in plant cells or organisms or occurs naturally therein only at a concentration which can essentially not cause any toxic effect. In the scope of the process of the invention, preference is given to applying the nontoxic substance X exogenously, for example via the medium or the growth substrate.

The term "compound capable of reducing the expression, amount, activity and/or function of at least one marker protein" is to be understood broadly and generally means any compounds which cause, directly or indirectly, alone or in cooperation with other factors, a reduction in the amount of protein, amount of RNA, gene activity, protein activity or protein function of at least one marker protein. Said compounds are also referred to under the generic term "anti-marker protein" compounds. The term "anti-marker

protein" compound includes in particular, but is not limited to, the nucleic acid sequences, ribonucleic acid sequences, double-stranded ribonucleic acid sequences, antisense ribonucleic acid sequences, expression cassettes, peptides, proteins or other factors used in the preferred embodiments within the scope of the process of the invention.

In a preferred embodiment, "anti-marker protein" compound means a DNA construct comprising

a) at least one expression cassette suitable for expressing a ribonucleic acid sequence and/or, if appropriate, a protein, said nucleic acid sequence and/or protein being capable of reducing the expression, amount, activity and/or function of the marker protein, or

b) at least one sequence which causes a partial or complete deletion or inversion of the sequence coding for said marker protein and thus enables the expression, amount, activity and/or function of the marker protein to be reduced, and also, if appropriate, further functional elements which facilitate and/or promote said deletion or inversion, or

c) at least one sequence which causes an insertion into the sequence coding for said marker protein and thus enables the expression, amount, activity and/or function of the marker protein to be reduced, and also, if appropriate, further functional elements which facilitate and/or promote said insertion.

The process of the invention stops the negative-selective action of the marker protein. To this extent, an "anti-marker protein" compound acts directly (e.g. via inactivation by means of insertion into the gene coding for the marker protein) or indirectly (e.g. by means of the ribonucleic acid sequence expressed via the expression cassette and/or, where appropriate, of the protein translated therefrom) as a positive selection marker. Hence, the selection system of the invention is to be referred to as a "reverse selection system", since it "reverts" the negative-selective action of the marker protein.

The process of the invention means a drastic broadening of the repertoire of positive selection processes for selecting transformed plant cells.

Another advantage is the fact that in a particular, preferred embodiment (e.g. via the action of a double-stranded or antisense RNA), it is possible to implement the selection effect without expressing a foreign protein (see below).

It is also advantageous that the marker protein used indirectly for selection (e.g. the negative selection marker) is not coupled genetically to the nucleic acid sequence to be inserted into the genome. In contrast to the otherwise customary selection processes, the marker protein, if it is a transgene, may be removed by simple segregation in the course of subsequent propagation or crossing.

"Plant cell" means within the scope of the present invention any type of cell which has been derived from a plant organism or is present therein. In this context, the term includes by way of example protoplasts, callus or cell cultures, microspores, pollen, cells in the form of tissues such as leaves, meristem, flowers, embryos, roots, etc. Included are, in particular, all of those cells and cell populations which are suitable as target tissues for a transformation.

In this context, "plant organism" comprises any organism capable of photosynthesis and also the cells, tissues, parts or propagation material (such as seeds or fruits) derived therefrom. Included within the scope of the invention are all genera and species of higher and lower plants of the plant kingdom. Preference is given to annual, perennial, monocotyledonous and dicotyledonous plants and also gymnosperms.

"Plant" means within the scope of the invention all genera and species of higher and lower plants of the plant kingdom. The term includes the mature plants, seed, shoots and seedlings, and also parts, propagation material (for example tubers, seeds or fruits), plant organs, tissues, protoplasts, callus and other cultures, for example cell cultures, derived therefrom, and also any other types of groupings of plant cells to give functional or structural units. Mature plants means plants at any developmental stage beyond that of the seedling. Seedling means a young immature plant at an early developmental stage. "Plant" comprises all annual and perennial monocotyledonous and dicotyledonous plants and includes by way of example but not by limitation those of the genera Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon,

Nicotiana, Solarium, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Picea and Populus.

Preference is given to plants of the following plant families: Amaranthaceae, Asteraceae, Brassicaceae, Carophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Leguminosae, Papilionoideae, Liliaceae, Linaceae, Malvaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Solanacea, Sterculiaceae, Tetragoniacea, Theaceae, Umbelliferae.

Preferred monocotyledonous plants are selected in particular from the monocotyledonous crop plants such as, for example, those in the family of Gramineae such as alfalfa, rice, corn, wheat or other cereal species such as barley, millet, rye, triticale or oats and also from sugar cane and all grass species.

Preferred dicotyledonous plants are selected in particular from the dicotyledonous crop plants such as, for example,

- Asteraceae, such as sunflower, tagetes or calendula and others,

- Compositae, in particular the genus Lactuca, very especially the species sativa (lettuce) and others,

- Cruciferae, especially the genus Brassica, very especially the species napus (oilseed rape), campestris (beet), oleracea cv Tastie (cabbage), oleracea cv Snowball Y (cauliflower) and oleracea cv Emperor (broccoli) and other cabbage species; and the genus Arabidopsis, very especially the species thaliana, and cress or canola and others,

- Cucurbitaceae, such as melon, pumpkin/squash or zucchini and others,

- Leguminosae, especially the genus Glycine, very especially the species max (soybean) and alfalfa, pea, bean plant or peanut, and others

- Rubiaceae, preferably the subclass Lamiidae, such as, for example, Coffea arabica or Coffea liberica (coffee bush) and others,

- Solanaceae, in particular the genus Lycopersicon, very especially the species esculentum (tomato), the genus Solanum, very especially the species tuberosum (potato) and melongena (eggplant), and the genus Capsicum, very especially the species annuum (pepper) and tobacco and others,

- Sterculiaceae, preferably the subclass Dilleniidae, such as, for example, Theobroma cacao (cacao tree) and others,

- Theaceae, preferably the subclass Dilleniidae, such as, for example, Camellia sinensis or Thea sinensis (tea shrub) and others,

- Umbelliferae, especially the genus Daucus (very especially the species carota (carrot)) and Apium (very especially the species graveolens dulce (celery)) and others,

and linseed, cotton, hemp, flax, cucumber, spinach, carrot, sugar beet and the various tree, nut and grapevine species, in particular banana and kiwi.

Plant organisms for the purposes of the invention are furthermore other photosynthetically active capable organisms such as, for example, algae, cyanobacteria and mosses. Preferred algae are green algae such as, for example, algae of the genus Haematococcus, Phaedactylum tricornatum, Volvox or Dunaliella. Particular preference is given to Synechocystis.

Particular preference is given to the group of plants, consisting of wheat, oats, millet, barley, rye, corn, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, oilseed rape, cress, Arabidopsis, cabbage species, soybean, alfalfa, pea, bean plants, peanut, potato, tobacco, tomato, eggplant, paprika, sunflower, tagetes, lettuce, calendula, melon, pumpkin and zucchini.

Most preference is given to

a) plants suitable for producing oil, such as, for example, oilseed rape, sunflower, sesame, safflower (Carthamus tinctorius), olive tree, soybean, corn, peanut, ricinus, oil palm, wheat, cacao tree or various nut species such as, for example, walnut, coconut or almond. Among these, particular preference is in turn given to dicotyledonous plants, in particular oilseed rape, soybean and sunflower.

b) plants suitable for producing starch, such as corn, wheat or potato, for example.

c) plants which are utilized as food and/or feedstuff and/or as useful plants and in which a resistance to pathogens would be advantageous, such as barley, rye, rice, potato, cotton, flax or linseed, for example.

d) plants which may be suitable for producing fine chemicals such as, for example, vitamins and/or carotenoids, such as oilseed rape, for example.

"Population of plant cells" means any group of plant cells, which may be subjected within the scope of the present invention to a transformation and from which transgenic plant cells transformed by the process of the invention may be obtained and isolated. In this context, said population may also be, for example, a plant tissue, organ or a cell culture, etc. Said population may comprise by way of example but not by limitation an isolated zygote, an isolated immature embryo, embryogenic callus, plant or else various flower tissues (both in vitro and in vivo).

"Genome" means the entirety of genetic information of a plant cell and comprises both genetic information of the nucleus and that of the plastids (e.g. chloroplasts) and mitochondria. However, genome preferably means the genetic information of the nucleus (for example of the nuclear chromosomes).

"Selection" means identifying and/or isolating successfully transformed plant cells from a population of nontransformed cells by using the process of the invention. This does not necessarily require that the selection be carried out directly with the transformed cells immediately after transformation. It is also possible to carry out the selection only at a later time, even with a later generation of the plant organisms (or cells, tissues, organs or propagation material derived therefrom) resulting from the transformation. Thus it is possible, for example, to transform Arabidopsis plants directly using, for example, the vacuum infiltration method (Clough S & Bent A (1998) Plant J 16(6):735-43; Bechtold N et al. (1993) CR Acad Sci Paris 1144(2):204-212), which subsequently produce transgenic seeds which may then be subjected to selection.

The fact that the nucleic acid sequence to be inserted is transformed "in combination with" the "anti-marker protein" compound (e.g. a DNA construct) is to be understood broadly and means that

at least one nucleic acid sequence to be inserted and at least one "anti-marker protein" compound are functionally coupled to one another so that the presence of the "anti-marker protein" compound in the plant cell, and of the selection advantage related thereto, indicates the parallel presence of the inserted nucleic acid sequence as likely. The nucleic acid sequence to be inserted and the "anti-marker protein" compound (e.g. a DNA construct) here may be, preferably but not necessarily, part of a single nucleic acid construct (e.g. a transformation construct or transformation vector), i.e. be present physicochemically coupled via a covalent bond. However, they may also be jointly introduced separately, for example in the course of a cotransformation, and exert their function within the scope of the process of the invention also in this way. In the case of the "anti-marker protein compound" acting via expressing an RNA (e.g. an antisense RNA or double-stranded RNA) or being such an RNA, "in combination" may also include those embodiments in which said RNA and the RNA expressed by the nucleic acid sequence inserted into the genome form an RNA strand.

"Nontoxic substance X" generally means substances which, compared to their reaction product Y, under otherwise identical conditions, have a reduced, preferably an essentially lacking biological activity, preferably toxicity. In this context, the toxicity of substance Y is at least twice as high as that of substance X, preferably at least five times as high, particularly preferably at least ten times as high, very particularly preferably at least twenty times as high, most preferably at least one hundred times as high. "Identical conditions" here means that all conditions are kept the same, apart from the different substances X and Y. Accordingly, identical molar concentrations of X and Y are used, with the medium, temperature, type of organism and density of organism, etc. being the same. The substance X may be converted to the substance Y in various ways, for example by hydrolysis, deamination, hydrolysis, dephosphorylation, phosphorylation, oxidation or any other type of activation, metabolization or conversion. The substance X may be, by way of example but not by limitation, the inactive precursor or derivative of a plant growth regulator or herbicide.

"Toxicity" or "toxic effect" means a measurable, negative influence on the physiology of the plant or of the plant cell and may comprise here symptoms such as, for example, but not limited thereto, a reduced or disrupted growth, a reduced or disrupted rate of photosynthesis, a reduced or disrupted cell division, a reduced or disrupted regeneration of a complete plant from cell culture or callus, etc.

The plant cells successfully transformed by means of the process of the invention may, to put it differently, have a growth advantage or selection advantage over the nontransformed cells of the same starting population under the influence of the substance "X". Growth or selection advantage is to be understood here broadly and means, for example, the fact that said transformed plant cells are capable of forming shoots and/or can be regenerated to give complete plants, whereas the nontransformed cells can do this only with a marked delay, if at all.

The term of "marker protein" is to be understood broadly and generally means all of those proteins which are capable of

i) exerting per se a toxic effect on the plant or plant cell, or

ii) converting directly or indirectly a nontoxic substance X into a substance Y which is toxic for the plant or plant cell.

In this context, the marker protein may be a plant-intrinsic, endogenous gene or else a transgene from a different organism. Preferably, the marker protein itself has no essential function for the organism including the marker protein. If the marker protein per se exerts a toxic effect, then it will preferably be expressed, for example, under an inducible promoter rather than constitutively.

Preferably, however, the marker protein converts directly or indirectly a nontoxic substance X into a substance Y which is toxic for the plant or plant cell. Particularly preferred marker proteins are the "negative selection markers" as are used, for example, in the course of targeted deletions from the genome.

Examples of marker proteins which may be mentioned but which are not limiting are:

(a) cytosine deaminases (CodA or CDase), with preference being given to using as the nontoxic substance X substances such as 5-fluorocytosine (5-FC). Cytosine deaminases catalyze the deamination of cytosine to give uracil (Kilstrup M et al. (1989) J Bacteriol 171:2124-2127; Anderson L et al. (1989) Arch Microbiol 152:115-118). Bacteria and fungi which have CDase activity convert 5-FC to the toxic metabolite ("Y") 5-fluorouracil (5-FU) (Polak A & Scholer HJ (1975) Chemotherapy (Basel) 21:113-130). 5-FC itself has low toxicity (Bennett JE, in Goodman and Gilman: the Pharmacological Basis

of Therapeutics. 8th ed., eds. Gilman AG et al. (Pergamon Press, New York) pp. 1165-1181). However, 5-FU has a highly cytotoxic effect, since it is subsequently metabolized to fluoro-UTP (FUTP) and fluoro-dUMP (FdUMP) and thus inhibits RNA and DNA synthesis (Calabrisi P & Chabner BA in Goodman and Gilman: the Pharmacological Basis of Therapeutics. 8th ed., eds. Gilman AG et al. (Pergamon Press, New York) pp. 1209-1263); Damon LE et al. (1989) Pharmac Ther 43:155-189).

Cells of higher plants and mammalian cells have no significant CDase activity and cannot deaminase 5-FC (Polak A et al. (1976) Chemotherapy 22:137-153; Koechlin BA et al. (1966) Biochemical Pharmacology 15:434-446). In this respect, the CDase is introduced as a transgene (e.g. in the form of a transgenic expression cassette) into plant organisms in the course of the process of the invention. Corresponding transgenic plant cells or organisms are then used as masterplants as starting material. Appropriate CDase sequences, transgenic plant organisms and the process of carrying out negative selection processes using, for example, 5-FC as nontoxic substance X, are known to the skilled worker (WO 93/01281; US 5,358,866; Gleave AP et al. (1999) Plant Mol Biol 40(2):223-35; Perera RJ et al. (1993) Plant Mol Biol 23(4):793-799; Stougaard J (1993) Plant J 3:755-761); EP-A1 595 837; Mullen CA et al. (1992) Proc Natl Acad Sci USA 89(1):33-37; Kobayashi T et al. (1995) Jpn J Genet 70(3):409-422; Schlaman HRM & Hooykaas PFF (1997) Plant J 11:1377-1385; Xiaohui Wang H et al. (2001) Gene 272(1-2): 249-255; Koprek T et al. (1999) Plant J 19(6):719-726; Gleave AP et al. (1999) Plant Mol Biol 40(2):223-235; Gallego ME (1999) Plant Mol Biol 39(1):83-93; Salomon S & Puchta H (1998) EMBO J 17(20):6086-6095; Thykjaer T et al. (1997) Plant Mol Biol 35(4):523-530; Serino G (1997) Plant J 12(3):697-701; Risseeuw E (1997) Plant J 11(4):717-728; Blanc V et al. (1996) Biochimie 78(6):511-517; Corneille S et al. (2001) Plant J 27:171-178). Cytosine deaminases and the genes coding therefor may be obtained from a multiplicity of organisms, preferably microorganisms such as, for example, the fungi Cryptococcus neoformans, Candida albicans, Torulopsis glabrata, Sporothrix schenckii, Aspergillus, Cladosporium and Phialophora (JE Bennett, Chapter 50: Antifungal Agents, in Goodman and Gilman's the Pharmacological Basis of Therapeutics 8th ed., A.G. Gilman, ed., Pergamon Press, New York, 1990) and the bacteria E.coli and Salmonella typhimurium (Andersen L et al. (1989) Arch Microbiol 152:115-118).

The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: S56903, and to the modified codA sequences described in EP-A1 595 873, which make expression in eukaryotes possible. Preference is given here to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 2 or, preferably, 4, in particular the sequences according to SEQ ID NO: 1 or, preferably, 3.

(b) cytochrome P-450 enzymes, in particular the bacterial cytochrome P-450 SU1 gene product (CYP105A1) from Streptomyces griseolus (strain ATCC 11796), with preference being given to using as nontoxic substance X substances such as the pro sulfonylurea herbicide R7402 (2-methylethyl-2-3-dihydro-N-[(4,6-dimethoxypyrimidin-2-yl)aminocarbonyl]-1,2-benzoisothiazole-7-sulfonamide 1,1-dioxide). Corresponding sequences and the process of carrying out negative selection processes using, for example, R7402 as nontoxic substance X are known to the skilled worker (O'Keefe DP et al. (1994) Plant Physiol 105:473-482; Tissier AF et al. (1999) Plant Cell 11:1841-1852; Koprek T et al. (1999) Plant J 19(6):719-726; O'Keefe DP (1991) Biochemistry 30(2):447-55). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: M32238. Preference is further given to nucleic acid sequences coding for the polypeptide according to SEQ ID NO: 6, in particular the sequence according to SEQ ID NO: 5.

(c) indoleacetic acid hydrolases such as, for example, Agrobacterium tumefaciens, tms2 gene product, with preference being given to using as nontoxic substance X substances such as auxin amide compounds or naphthaleneacetamide (NAM) (with NAM being converted to naphthaleneacetic acid, a phytotoxic substance). Corresponding sequences and the process of carrying out negative selection processes using, for example, NAM as nontoxic substance X are known to the skilled worker (Fedoroff NV & Smith DL (1993) Plant J 3:273-289; Upadhyaya NM et al. (2000) Plant Mol Biol Rep 18:227-223; Depicker AG et al. (1988) Plant Cell rep 104:1067-1071; Karlin-Neumannn GA et al. (1991) Plant Cell 3:573-582; Sundaresan V et al. (1995) Gene Develop 9:1797-1810; Cecchini E et al. (1998) Mutat Res 401(1-2):199-206; Zubko E et al. (2000) Nat Biotech-

nol 18:442-445). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: NC_003308 (Protein_id="NP_536128.1), AE009419, AB016260 (Protein_id="BAA87807.1) and NC002147. Preference is further given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 8 or 10, in particular the sequences according to SEQ ID NO: 7 or 9.

(d) haloalkane dehalogenases (dhlA gene product), for example from Xanthobacter autotropicus GJ10. The dehalogenase hydrolyzes dihaloalkanes such as 1,2-dichloroethane (DCE) to give halogenated alcohols and inorganic halides (Naested H et al. (1999) Plant J 18(5)571-576; Janssen DB et al. (1994) Annu Rev Microbiol 48: 163-191; Janssen DB (1989) J Bacteriol 171(12):6791-9). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: M26950. Preference is further given to nucleic acid sequences coding for the polypeptide according to SEQ ID NO: 12, in particular the sequence according to SEQ ID NO: 11.

(e) thymidine kinases (TK), in particular viral TKs from viruses such as Herpes simplex virus, SV40, cytomegalovirus, Varicella zoster virus, in particular the TK of Herpes simplex virus type 1 (TK HSV-1), with preference being given to using as nontoxic substance X substances such as Acyclovir, Ganciclovir or 1,2-deoxy-2-fluoro-β-D-arabinofuranosil-5-iodouracil (FIAU). Corresponding sequences and the process of carrying out negative selection processes using, for example, Acyclovir, Ganciclovir or FIAU as nontoxic substance X are known to the skilled worker (Czako M & Marton L (1994) Plant Physiol 104:1067-1071; Wigler M et al. (1977) Cell 11(1):223-232; McKnight SL et al. (1980) Nucl Acids Res 8(24):5949-5964; McKnight SL et al. (1980) Nucl Acids Res 8(24):5931-5948; Preston et al. (1981) J Virol 38(2):593-605; Wagner et al. (1981) Proc Natl Acad Sci USA 78(3):1441-1445; St. Clair et al.(1987) Antimicrob Agents Chemother 31(6):844-849). The sequences, materials and processes disclosed in the context of said publications are hereby explic-

itly referred to.

Particular preference is given to sequences according to GenBank Acc. No: J02224, V00470 and V00467. Preference is also given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 14 or 16, in particular the sequences according to SEQ ID NO: 13 or 15.

(f) guanine phosphoribosyl transferases, hypoxanthine phosphoribosyl transferases or xanthine guanine phosphoribosyl transferases, with preference being given to using as nontoxic substance X substances such as 6-thioxanthine or allopurinol. Preference is given to guanine phosphoribosyl transferases (gpt), for example from E. Coli (Besnard et al. (1987) Mol Cell Biol 7:4139; Mzoz and Moolten (1993) Human Gene Therapy 4:589-595; Ono et al. (1997) Hum Gene Ther 8(17):2043-55), hypoxanthine phosphoribosyl transferases (HPRT; Jolly et al. (1983) Proc Natl Acad Sci USA 80:477; Fonwick "The HGPRT Systern", pp. 333-373, M. Gottesman (ed.), Molecular Cell Genetics, John Wiley and Sons, New York, 1985), xanthine guanine phosphoribosyl transferases, for example from Toxoplasma gondii (Knoll LJ et al.(1998) Mol Cell Biol 18(2):807-814; Donald RG et al. (1996) J Biol Chem 271(24):14010-14019). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: U10247 (Toxoplasma gondii HXGPRT), M13422 (E. coli gpt) and X00221 (E. coli gpt). Preference is also given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 18, 20 or 22, in particular the sequences according to SEQ ID NO: 17, 19 or 21.

(g) purine nucleoside phosphorylases (PNP; DeoD gene product), for example from E. coli, with preference being given to using as nontoxic substance X substances such as 6-methylpurine deoxyribonucleoside. Corresponding sequences and the process of carrying out negative selection processes using, for example, 6-methylpurine deoxyribonucleoside as nontoxic substance X are known to the skilled worker (Sorscher EJ et al. (1994) Gene Therapy 1:233-238). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: M60917. Preference is also given to nucleic

acid sequences coding for the polypeptide according to SEQ ID NO: 24, in particular the sequence according to SEQ ID NO: 23.

h) phosphonate monoester hydrolases which convert inactive ester derivatives of the herbicide glyphosate (e.g. glycerylglyphosate) into the active form of the herbicide. Corresponding sequences and the process of carrying out negative selection processes using, for example, glycerylglyphosate are known to the skilled worker (US 5,254,801; Dotson SB et al. (1996) Plant J 10(2):383-392; Dotson SB et al. (1996) J Biol Chem 271(42): 25754-25761). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: U44852. Preference is also given to nucleic acid sequences coding for the polypeptide according to SEQ ID NO: 26, in particular the sequence according to SEQ ID NO: 25.

(i) aux-1 and, preferably, aux-2 gene products, for example of the Ti plasmids of Agrobacterium strains such as A.rhizogenes or A.tumefaciens (Beclin C et al. (1993) Transgenics Res 2:4855); Gaudin V, Jouanin L. (1995) Plant Mol Biol. 28(1):123-36.

The activity of the two enzymes causes the plant cell to produce indoleacetamide (IAA). Aux-1 encodes an indoleacetamide synthase (IAMS) and converts tryptophan into indoleacetamide (VanOnckelen et al. (1986) FEBS Lett. 198: 357-360). Aux-2 encodes the enzyme indoleacetamide hydrolase (IAMH) and converts indoleacetamide, a substance without phytohormone activity, into the active auxin indoleacetic acid (Inze D et al. (1984) Mol Gen Genet 194:265-274; Tomashow et al. (1984) Proc Natl Acad Sci USA 81:5071-5075; Schroder et al. (1984) Eur J Biochem 138:387-391). The enzyme IAMH may also hydrolyze a number of indoleamide substrates such as, for example, naphthaleneacetamide, the latter being converted into the plant growth regulator naphthaleneacetic acid (NAA). The use of the IAMH gene as a negative selection marker is described, for example, in US 5,180,873. Corresponding enzymes have also been described in A. rhizogenes, A. vitis (Canaday J et al. (1992) Mol Gen Genet 235:292-303) and Pseudomonas savastanoi (Yamada et al. (1985) Proc Natl Acad Sci USA 82:6522-6526). The use as a negative selection marker for destroying partic-

ular cell tissues (e.g. pollen; US 5,426,041) or transgenic plants (US 5,180,873) has been described. Corresponding sequences and the process of carrying out negative selection processes using, for example, naphthaleneacetamide are known to the skilled worker (see above). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to the GenBank Acc. No: M61151, AF039169 and AB025110. Preference is also given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 28, 30, 32, 34 or 36, in particular the sequences according to SEQ ID NO: 27, 29, 31, 33 or 35.

(j) adenine phosphoribosyl transferases (APRT), with preference being given to using as nontoxic substance X substances such as 4-aminopyrazolopyrimidine. Corresponding sequences and the process of carrying out negative selection processes with use are known to the skilled worker (Wigler M et al. (1979) Proc Natl Acad Sci USA 76(3):1373-6; Taylor et al. "The APRT Systern", pp., 311-332, M. Gottesman (ed.), Molecular Cell Genetics, John Wiley and Sons, New York, 1985).

k) methoxinine dehydrogenases, with preference being given to using as nontoxic substance X substances such as 2-amino-4-methoxybutanoic acid (methoxinine) which is converted into the toxic methoxyvinyl glycine (Margraff R et al. (1980) Experimentia 36: 846).

l) rhizobitoxin synthases, with preference being given to using as nontoxic substance X substances such as 2-amino-4-methoxybutanoic acid (methoxinine) which is converted into the toxic 2-amino-4-[2-amino-3-hydroxypropyl]-trans-3-butanoic acid (rhizobitoxin) (Owens LD et al. (1973) Weed Science 21:63-66),

m) 5-methylthioribose (MTR) kinases, with preference being given to using as nontoxic substance X substances such as 5-(trifluoromethyl)thioribose (MTR analog, "subversive substrate") which is converted, via an unstable intermediate, into the toxic substance (Y) carbothionyl difluoride. The MTR kinase is a key enzyme of the methionine salvage pathway. Corresponding enzyme activities have been described in plants, bacteria and protozoa but not in mammals. MTR kinases of various species have been identified owing to defined sequence motifs (Sekowska A et al. (2001) BMC Microbiol 1:15;

http://www.biomedcentral.com/1471-2180/1/15). Corresponding sequences and the process of carrying out negative selection processes using, for example, 5-(trifluoromethyl)thioribose are known to the skilled worker and readily obtainable from the appropriate sequence database (e.g. GenBank) (Sekowska A et al. (2001) BMC Microbiol 1:15; Cornell KA et al. (1996) 317:285-290). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

However, a plant MTR kinase has not yet been identified unambiguously and is provided within the scope of the process of the invention (SEQ ID NO: 39 and, respectively, 40). In addition, homologs from other plant species are provided, namely from corn (SEQ ID NO: 59 and, respectively, 60), oilseed rape (SEQ ID NO: 61, 63 and, respectively, 62, 64), rice (SEQ ID NO: 65 and, respectively, 66) and soybean (SEQ ID NO: 67 and, respectively, 68).

Accordingly, the invention further relates to amino acid sequences encoding a plant 5-methylthioribose kinase, wherein said amino acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 60, 62, 64, 66 or 68.

Accordingly, the invention further relates to nucleic acid sequences encoding a plant 5-methylthioribose kinase, wherein said nucleic acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 59, 61, 63, 65 or 67. Even if said sequences are in parts only fragments of complete cDNAs, their length is nevertheless more than sufficient in order to ensure a use and functionality as antisense RNA or double-stranded RNA. Preference is given to using as marker protein a plant endogenous MTR kinase. Further endogenous plant MTR kinases may readily be identified by means of screening databases or gene libraries using conserved, MTK kinase-typical motifs. Said motifs may be derived from Fig. 9a-b, for example. Such motifs may comprise, by way of example but not by limitation, the following sequences:

E(V/I)GDGN(L/I)N(L/Y/F)V(F/Y), preferably EVGDGNLN(Y/F)V(F/Y)
KQALPY(V/I)RC
SWPMT(R/K)ERAYF
PEVYHFDRT
GMRY(I/L)EPPHI
CRLTEQVVFSDPY
HGDLH(S/T)GS

Further suitable motifs may be derived from Fig. 9a-b without difficulty.

Particular preference is given to sequences according to GenBank Acc. No: AF212863 or AC079674 (Protein_ID=AAG51775.1). Preference is also given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 38 or 40, in particular the sequences according to SEQ ID NO: 37 or 39.

n) alcohol dehydrogenases (Adh), in particular plant Adh-1 gene products, with preference being given to using as nontoxic substance X substances such as allyl alcohol which is converted in this manner into the toxic substance (Y) acrolein. Corresponding sequences and the process of carrying out negative selection processes using, for example, allyl alcohol are known to the skilled worker and readily obtainable from the appropriate sequence database (e.g. GenBank) (Wisman E et al. (1991) Mol Gen Genet 226(1-2):120-8; Jacobs M et al. (1988) Biochem Genet 26(1-2):105-22; Schwartz D. (1981) Environ Health Perspect 37:75-7). The sequences, materials and processes disclosed in the context of said publications are hereby explicitly referred to.

Particular preference is given to sequences according to GenBank Acc. No: X77943, M12196, AF172282, X04049 or AF253472. Preference is also given to nucleic acid sequences coding for polypeptides according to SEQ ID NO: 42, 44, 46 or 48, in particular the sequences according to SEQ ID NO: 41, 43, 45 or 47.

(o) Further suitable negative selection markers are those sequences which exert per se a toxic action on plant cells, such as, for example, diphtheria toxin A, ribonucleases such as barnase and also ribosome-inhibiting proteins such as ricin. In this context, these proteins are preferably expressed in the plant cells inducibly rather than constitutively. The induction is preferably carried out chemically, it being possible, for example, to use the chemically inducible promoters mentioned below in order to ensure said chemically induced expression.

"Reduction" or "to reduce" is to be interpreted broadly in connection with a marker protein or with its amount, expression, activity and/or function and comprises the partial or essentially complete stopping or blocking, based on different cell-biological

mechanisms, of the functionality of a marker protein in a plant cell, plant or a part, tissue, organ, cells or seeds derived therefrom.

A reduction for the purpose of the invention also comprises a reduction of the amount of a marker protein down to an essentially complete lack of said marker protein (i.e. a lack of detectability of marker protein activity or marker protein function or a lack of immunological detectability of said marker protein). In this context, expression of a particular marker protein (or of its amount, expression, activity and/or function) in a cell or an organism is reduced preferably by more than 50%, particularly preferably by more than 80%, very particularly preferably by more than 90%, most preferably by more than 98%. Reduction means in particular also the complete lack of the marker protein (or of its amount, expression, activity and/or function). In this context, activity and/or function mean preferably the property of the marker protein of exerting a toxic effect on the plant cell or the plant organism and, respectively, the ability to convert the substance X into the substance Y. The toxic effect caused by the marker protein is reduced preferably by more than 50%, particularly preferably by more than 80%, very particularly preferably by more than 90%, most preferably by more than 98%. "Reduction" includes of course within the scope of the present invention also a complete, 100% reduction or removal of the marker protein (or of its amount, expression, activity and/or function) (for example by deleting the marker protein gene from the genome).

The invention comprises various strategies for reducing the expression, amount, activity and/or function of the marker protein. The skilled worker appreciates the fact that a number of various methods are available in order to influence the expression, amount, activity and/or function of a marker protein in the desired way. Examples which may be mentioned but which are not limiting are:

a) introducing at least one marker protein double-stranded ribonucleic acid sequence (MP-dsRNA) or an expression cassette or expression cassettes ensuring expression thereof. Included are those processes in which the MP-dsRNA is directed against a marker protein gene (i.e. genomic DNA sequences such as promoter sequences) or a marker protein gene transcript (i.e. mRNA sequences).

b) introducing at least one marker protein antisense ribonucleic acid sequence (MP-antisenseRNA) or an expression cassette ensuring expression thereof. Included are those processes in which the MP-antisenseRNA is directed against a marker protein gene (i.e. genomic DNA sequences) or a marker protein gene transcript (i.e. RNA sequences). α-anomeric nucleic acid sequences are also included.

c) introducing at least one MP-antisenseRNA combined with a ribozyme or an expression cassette ensuring expression thereof

d) introducing at least one marker protein sense ribonucleic acid sequence (MP-senseRNA) for inducing a cosuppression or an expression cassette ensuring expression thereof

e) introducing at least one DNA- or protein-binding factor against a marker protein gene, marker protein RNA or marker protein or an expression cassette ensuring expression thereof

f) introducing at least one viral nucleic acid sequence causing degradation of the marker protein RNA or an expression cassette ensuring expression thereof

g) introducing at least one construct for generating a functional loss (e.g. generation of stop codons, shifts in the reading frame etc.) on a marker protein gene, for example by generating an insertion, deletion, inversion or mutation in a marker protein gene. Preferably, knockout mutants may be generated by means of targeted insertion into said marker protein gene via homologous recombination or by introducing sequence-specific nucleases against marker protein gene sequences.

It is known to the skilled worker that it is also possible to use other processes within the scope of the present invention in order to reduce a marker protein or its activity or function. For example, it may also be advantageous, depending on the type of the marker protein used, to introduce a dominant-negative variant of a marker protein or an expression cassette ensuring expression thereof. In this context, any single one of these processes may cause a reduction in the expression, amount, activity and/or function of a marker protein. A combined application is also conceivable. Further methods are known to the skilled worker and may comprise hindering or stopping the processing of the marker protein, the transport of the marker protein or of its mRNA, the inhibition of ribosome attachment, the inhibition of RNA splicing,

the induction of an enzyme degrading marker protein RNA and/or the inhibition of translational elongation or termination.

The embodiments below will describe by way of example the individual preferred processes:

a) Introducing a double-stranded ribonucleic acid sequence of a marker protein (MP-dsRNA)

The process of gene regulation by means of double-stranded RNA ("double-stranded RNA interference"; dsRNAi) has been described many times for animal and plant organisms (e.g. Matzke MA et al. (2000) Plant Mol Biol 43:401-415; Fire A. et al (1998) Nature 391:806-811; WO 99/32619; WO 99/53050; WO 00/68374; WO 00/44914; WO 00/44895; WO 00/49035; WO 00/63364). The processes and methods described in the references indicated are hereby explicitly referred to. dsRNAi processes are based on the phenomenon that simultaneously introducing the complementary strand and contour strand of a gene transcript suppresses expression of the corresponding gene in a highly efficient manner. Preferably, the phenotype caused is very similar to that of a corresponding knockout mutant (Waterhouse PM et al. (1998) Proc Natl Acad Sci USA 95:13959-64). The dsRNAi process has proved to be particularly efficient and advantageous in reducing marker protein expression.

Double-stranded RNA molecule means within the scope of the invention preferably one or more ribonucleic acid sequences which, owing to complementary sequences, are theoretically (e.g. according to the base pair rules by Watson and Crick) and/or actually (e.g. owing to hybridization experiments in vitro and/or in vivo) capable of forming double-stranded RNA structures. The skilled worker is aware of the fact that the formation of double-stranded RNA structures represents a state of equilibrium. Preferably, the ratio of double-stranded molecules to corresponding dissociated forms is at least 1 to 10, preferably 1:1, particularly preferably 5:1, most preferably 10:1.

The invention therefore further relates to double-stranded RNA molecules (dsRNA-Moleküle) which, when introduced into a plant organism (or into a cell, tissue, organ or propagation material derived therefrom) cause the reduction of at least one marker protein. The double-stranded RNA molecule for reducing expression of a marker protein (MP-dsRNA) here preferably comprises

a) a "sense" RNA strand comprising at least one ribonucleotide sequence which is essentially identical to at least a part of

the "sense" RNA transcript of a nucleic acid sequence coding for a marker protein, and

b) an "antisense" RNA strand which is essentially, preferably fully, complementary to the RNA sense strand under a).

With respect to the dsRNA molecules, marker protein nucleic acid sequence preferably means a sequence according to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47 or a functional equivalent thereof.

"Essentially identical" means that the dsRNA sequence may also have insertions, deletions and also individual point mutations in comparison with the marker protein target sequence and nevertheless causes an efficient reduction in expression. The homology (as defined hereinbelow) between the "sense" strand of an inhibitory dsRNA and at least one part of the "sense" RNA transcript of a nucleic acid sequence coding for a market protein (or between the "antisense" strand of the complementary strand of a nucleic acid sequence coding for a marker protein) is preferably at least 75%, preferably at least 80%, very particularly preferably at least 90%, most preferably 100%.

A 100% sequence identity between dsRNA and a marker protein gene transcript is not absolutely necessary in order to cause an efficient reduction in marker protein expression. Consequently, the process is advantageously tolerant toward sequence deviations as may be present due to genetic mutations, polymorphisms or evolutionary divergences. Thus it is possible, for example, using the dsRNA which has been generated starting from the marker protein sequence of the first organism, to suppress marker protein expression in a second organism. This is particularly advantageous when the marker protein used is a plant-intrinsic, endogenous marker protein (for example a 5-methylthioribose kinase or alcohol dehydrogenase). For this purpose, the dsRNA preferably includes sequence regions of marker protein gene transcripts which correspond to conserved regions. Said conserved regions may be readily derived from sequence comparisons.

The length of the subsection is at least 10 bases, preferably at least 25 bases, particularly preferably at least 50 bases, very particularly preferably at least 100 bases, most preferably at least 200 bases or at least 300 bases.

Alternatively, an "essentially identical" dsRNA may also be defined as a nucleic acid sequence capable of hybridizing with part of a marker protein gene transcript (e.g. in 400 mM NaCl, 40 mM

PIPES pH 6.4, 1 mM EDTA at 50°C or 70°C for 12 to 16 h).

"Essentially complementary" means that the "antisense" RNA strand may also have insertions, deletions and also individual point mutations in comparison with the complement of this "sense" RNA strand. The homology between the "antisense" RNA strand and the complement of the "sense" RNA strand is preferably at least 80%, preferably at least 90%, very particularly preferably at least 95%, most preferably 100%.

"Part of the "sense" RNA transcript" of a nucleic acid sequence coding for a marker protein means fragments of an RNA or mRNA transcribed or transcribable from a nucleic acid sequence coding for a marker protein, preferably from a marker protein gene. In this context, the fragments have a sequence length of preferably at least 20 bases, preferably at least 50 bases, particularly preferably at least 100 bases, very particularly preferably at least 200 bases, most preferably at least 500 bases. The complete transcribable RNA or mRNA is also included. Included are also sequences such as those which may be transcribed under artificial conditions from regions of a marker protein gene which are otherwise, under natural conditions, not transcribed, such as promoter regions, for example.

The dsRNA may consist of one or more strands of polyribonucleotides. Naturally, in order to achieve the same purpose, it is also possible to introduce a plurality of individual dsRNA molecules which comprise in each case one of the above-defined ribonucleotide sequence sections into the cell or the organism. The double-stranded dsRNA structure may be formed starting from two complementary, separate RNA strands or, preferably, starting from a single, self-complementary RNA strand. In this case, the "sense" RNA strand and the "antisense" RNA strand are preferably connected covalently to one another in the form of an inverted "repeat".

As described in WO 99/53050, for example, the dsRNA may also comprise a hairpin structure by connecting the "sense" and the "antisense" strands by a connecting sequence ("linker"; for example an intron). Preference is given to the self-complementary dsRNA structures, since they require only the expression of an RNA sequence and always comprise the complementary RNA strands in an equimolar ratio. The connecting sequence may is preferably an intron (e.g. an intron of the potato ST-LS1 gene; Vancanneyt GF et al. (1990) Mol Gen Genet 220(2):245-250).

The nucleic acid sequence coding for a dsRNA may include further elements such as, for example, transcription termination signals or polyadenylation signals.

Bringing together, if intended, the two strands of the dsRNA in a cell or plant may be achieved by way of example in the following way:

a) transformation of the cell or plant with a vector comprising both expression cassettes,

b) cotransformation of the cell or plant with two vectors, one of which comprises the expression cassettes containing the "sense" strand and the other one of which comprises the expression cassettes containing the "antisense" strand.

The formation of the RNA duplex may be initiated either outside or inside the cell.

The dsRNA may be synthesized either in vivo or in vitro. For this purpose, a DNA sequence coding for a dsRNA may be inserted into an expression cassette under the control of at least one genetic control element (such as a promoter, for example). A polyadenylation is not necessary and neither need any elements for initiating a translation be present. Preference is given to the expression cassette for the MP-dsRNA being present on the transformation construct or the transformation vector. For this purpose, the expression cassettes coding for the "antisense" strand and/or the "sense" strand of an MP-dsRNA or for the self-complementary strand of the dsRNA are preferably inserted into a transformation vector and introduced into the plant cell by using the processes described below. A stable insertion into the genome may be advantageous for the process of the invention but is not absolutely necessary. Since a dsRNA causes a long-term effect, transient expression is also sufficient in many cases. The dsRNA may also be part of the RNA to be expressed by the nucleic acid sequence to be inserted by fusing it, for example, to the 3'-untranslated part of said RNA.

The dsRNA may be introduced in an amount which makes possible at least one copy per cell. Higher amounts (e.g. at least 5, 10, 100, 500 or 1000 copies per cell) may, if appropriate, cause a more efficient reduction.

b) Introducing an antisense ribonucleic acid sequence of a marker protein (MP-antisenseRNA)

Processes for reducing a particular protein by means of the "antisense" technique have been described multiple times, also in plants (Sheehy et al. (1988) Proc Natl Acad Sci USA 85: 8805-8809; US 4,801,340; Mol JN et al. (1990) FEBS Lett 268(2):427-430). The antisense nucleic acid molecule hybridizes or binds to the cellular mRNA and/or genomic DNA coding for the marker protein to be reduced, thereby suppressing transcription and/or translation of said marker protein. The hybridization may be produced in a conventional manner via the formation of a stable duplex or, in the case of genomic DNA, by binding of the antisense nucleic acid molecule to the duplex of the genomic DNA via specific interaction in the large groove of the DNA helix.

An MP-antisenseRNA may be derived using the nucleic acid sequence coding for this marker protein, for example the nucleic acid sequence according to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47 according to the base pair rules by Watson and Crick. The MP-antisenseRNA may be complementary to the entire transcribed mRNA of the marker protein, may be limited to the coding region or may consist only of an oligonucleotide which is complementary to a part of the coding or noncoding sequence of the mRNA. Thus, for example, the oligonucleotide may be complementary to the region comprising the translation start site for the marker protein. The MP-antisenseRNA may be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length, but may also be longer and comprise at least 100, 200, 500, 1000, 2000 or 5000 nucleotides. MP-antisenseRNA are preferably expressed recombinantly in the target cell in the course of the process of the invention.

The MP-antisenseRNA may also be part of an RNA to be expressed by the nucleic acid sequence to be inserted by being fused, for example, to the 3'-untranslated part of said RNA.

The invention further relates to transgenic expression cassettes containing a nucleic acid sequence coding for at least part of a marker protein, with said nucleic acid sequence being functionally linked in antisense orientation to a promoter functional in plant organisms. Said expression cassettes may be part of a transformation construct or transformation vector or else may be introduced in the course of a cotransformation.

In a further preferred embodiment, expression of a marker protein may be inhibited by nucleotide sequences which are complementary to the regulatory region of a marker protein gene (e.g. a marker protein promoter and/or enhancer) and which form with the DNA double helix there triple-helical structures, thereby reducing transcription of the marker protein gene. Corresponding processes

have been described (Helene C (1991) Anticancer Drug Res 6(6):569-84; Helene C et al. (1992) Ann NY Acad Sci 660:27-36; Maher LJ (1992) Bioassays 14(12):807-815).

In a further embodiment, the MP-antisenseRNA may be an α-anomeric nucleic acid. Such α-anomeric nucleic acid molecules form with complementary RNA specific double-stranded hybrids in which, in contrast to the conventional β-nucleic acids, the two strands are oriented parallel to one another (Gautier C et al. (1987) Nucleic Acids Res 15:6625-6641).

c) Introducing an MP-antisenseRNA combined with a ribozyme

Advantageously, the above-described antisense strategy may be coupled to a ribozyme process. Catalytic RNA molecules or ribozymes may be adapted to any target RNA and cleave the phosphodiester backbone in specific positions, thereby functionally deactivating said target RNA (Tanner NK (1999) FEMS Microbiol Rev 23(3):257-275). In the process, the ribozyme is not modified itself but is capable of cleaving in an analogous manner further target RNA molecules, thereby acquiring the properties of an enzyme. The incorporation of ribozyme sequences into "antisense" RNAs imparts specifically to these "antisense" RNAs this enzyme-like, RNA-cleaving property and thus increases their efficiency in inactivating the target RNA. The preparation and use of appropriate ribozyme "antisense" RNA molecules have been described (inter alia in Haseloff et al. (1988) Nature 334: 585-591); Haselhoff and Gerlach (1988) Nature 334:585-591; Steinecke P et al. (1992) EMBO J 11(4):1525- 1530; de Feyter R et al. (1996) Mol Gen Genet. 250(3):329-338).

In this way, it is possible to use ribozymes (e.g. hammerhead ribozymes; Haselhoff and Gerlach (1988) Nature 334:585-591) in order to catalytically cleave the mRNA of a marker protein to be reduced and thus prevent translation. The ribozyme technique may increase the efficiency of an antisense strategy. Processes for expressing ribozymes in order to reduce particular proteins have been described in (EP 0 291 533, EP 0 321 201, EP 0 360 257). Ribozyme expression has likewise been described in plant cells (Steinecke P et al. (1992) EMBO J 11(4):1525-1530; de Feyter R et al. (1996) Mol Gen Genet. 250(3):329-338). Suitable target sequences and ribozymes may be determined, for example, as described in "Steinecke P, Ribozymes, Methods in Cell Biology 50, Galbraith et al. eds, Academic Press, Inc. (1995), pp. 449-460", by calculating the secondary structures of ribozyme RNA and target RNA and by the interaction thereof (Bayley CC et al. (1992) Plant Mol Biol. 18(2):353-361; Lloyd AM and Davis RW et al. (1994) Mol Gen Genet. 242(6):653-657). It is possible, for exam-

ple, to construct derivatives of the Tetrahymena L-19 IVS RNA which have regions complementary to the mRNA of the marker protein to be suppressed (see also US 4,987,071 and US 5,116,742). Alternatively, such ribozymes may also be identified via a selection process from a library of various ribozymes (Bartel D and Szostak JW (1993) Science 261:1411-1418).

d) Introducing a sense ribonucleic acid sequence of a marker protein (MP-senseRNA) for inducing a cosuppression

Expression of a marker protein ribonucleic acid sequence (or a part thereof) in sense orientation may result in a cosuppression of the corresponding marker protein gene. Expression of sense RNA with homology to an endogenous marker protein gene may reduce or switch off expression of the latter, as has been described similarly for antisense approaches (Jorgensen et al. (1996) Plant Mol Biol 31(5):957-973; Goring et al. (1991) Proc Natl Acad Sci USA 88:1770-1774; Smith et al. (1990) Mol Gen Genet 224:447-481; Napoli et al. (1990) Plant Cell 2:279-289; Van der Krol et al. (1990) Plant Cell 2:291-99). In this context, the introduced construct may represent completely or only partially the homologous gene to be reduced. The possibility of translation is not required. The application of this technique to plants has been described (e.g. Napoli et al. (1990) Plant Cell 2:279-289; in US 5,034,323.

The cosuppression is preferably carried out using a sequence which is essentially identical to at least part of the nucleic acid sequence coding for a marker protein, for example the nucleic acid sequence according to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 ,31, 33, 35, 37, 39, 41, 43, 45 or 47.

The MP-senseRNA is preferably chosen in such a way that a translation of the marker protein or a part thereof cannot occur. For this purpose, for example, the 5'-untranslated or 3'-untranslated region may be chosen or else the ATG start codon may be deleted or mutated.

e) Introducing DNA- or protein-binding factors against marker protein genes, marker protein RNAs or proteins

Marker protein expression may also be reduced using specific DNA-binding factors, for example factors of the zinc finger transcription factor type. These factors attach to the genomic sequence of the endogenous target gene, preferably in the

regulatory regions, and cause a reduction in expression. Appropriate processes for preparing corresponding factors have been described (Dreier B et al. (2001) J Biol Chem 276(31):29466-78; Dreier B et al. (2000) J Mol Biol 303(4):489-502; Beerli RR et al. (2000) Proc Natl Acad Sci USA 97 (4):1495-1500; Beerli RR et al. (2000) J Biol Chem 275(42):32617-32627; Segal DJ and Barbas CF 3rd. (2000) Curr Opin Chem Biol 4(1):34-39; Kang JS and Kim JS (2000) J Biol Chem 275(12):8742-8748; Beerli RR et al. (1998) Proc Natl Acad Sci USA 95(25):14628- 14633; Kim JS et al. (1997) Proc Natl Acad Sci USA 94(8):3616 -3620; Klug A (1999) J Mol Biol 293(2):215-218; Tsai SY et al. (1998) Adv Drug Deliv Rev 30(1-3):23-31; Mapp AK et al. (2000) Proc Natl Acad Sci USA 97(8):3930-3935; Sharrocks AD et al. (1997) Int J Biochem Cell Biol 29(12):1371-1387; Zhang L et al. (2000) J Biol Chem 275(43):33850-33860).

These factors may be selected using any segment of a marker protein gene. This section is preferably in the region of the promoter region. However, for gene suppression, it may also be in the region of the coding exons or introns.

It is also possible to introduce factors which inhibit the marker protein itself into a cell. These protein-binding factors may be, for example, aptamers (Famulok M and Mayer G (1999) Curr Top Microbiol Immunol 243:123-36) or antibodies or antibody fragments or single-chain antibodies. Obtaining these factors has been described (Owen M et al. (1992) Biotechnology (N Y) 10(7):790-794; Franken E et al. (1997) Curr Opin Biotechnol 8(4):411-416; Whitelam (1996) Trend Plant Sci 1:286-272).

f) Introducing viral nucleic acid sequences and expression constructs causing the degradation of marker protein RNA

Marker protein expression may also be effectively implemented by inducing the specific degradation of marker protein RNA by the plant with the aid of a viral expression system (Amplikon; Angell SM et al. (1999) Plant J 20(3):357-362). These systems, also referred to as "VIGS" (viral induced gene silencing), introduce nucleic acid sequences with homology to the transcript of a marker protein to be reduced into the plant by means of viral vectors. Transcription is then switched off, presumably mediated by plant defence mechanisms against viruses. Appropriate techniques and processes have been described (Ratcliff F et al. (2001) Plant J 25(2):237-45; Fagard M und Vaucheret H (2000) Plant Mol Biol 43(2-3):285-93; Anandalakshmi R et al. (1998) Proc Natl Acad Sci USA 95(22):13079-84; Ruiz MT (1998) Plant Cell 10(6):937-46).

VIGS-mediated reduction is preferably implemented using a sequence which is essentially identical to at least part of the nucleic acid sequence coding for a marker protein, for example the nucleic acid sequence according to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47.

g) Introducing constructs for generating a functional loss or a functional reduction of marker protein genes

The skilled worker knows numerous possible processes of how to modify genomic sequences in a targeted manner. These include, in particular, processes such as the generation of knockout mutants by means of targeted homologous recombination, for example by generating stop codons, shifts in the reading frame etc. (Hohn B and Puchta H (1999) Proc Natl Acad Sci USA 96:8321-8323) or the targeted deletion or inversion of sequences by means of, for example, sequence-specific recombinases or nucleases (see below).

In a preferred embodiment, the marker protein gene is inactivated by introducing a sequence-specific recombinase. Thus it is possible, for example, for the marker protein gene to include recognition sequences for sequence-specific recombinases or to be flanked by such sequences, and introducing the recombinase then deletes or inverts particular sequences of the marker protein gene, thus leading to inactivation of the marker protein gene. A corresponding procedure is depicted diagrammatically in Fig. 1.

Appropriate processes for deletion/inversion of sequences by means of sequence-specific recombinase systems are known to the skilled worker. Examples which may be mentioned are the Cre/lox system of bacteriophage P1 (Dale EC and Ow DW (1991) Proc Natl Acad Sci USA 88:10558-10562; Russell SH et al. (1992) Mol Gen Genet 234:49-59; Osborne BI et al. (1995) Plant J 7:687-701), the yeast FLP/FRT system (Kilby NJ et al. (1995) Plant J 8:637-652; Lyznik LA et al. (1996) Nucl Acids Res 24:3784-3789), the Gin recombinase of the Mu phage, the E. coli Pin recombinase and the R/RS system of the pSR1 plasmids (Onouchi H et al.(1995) Mol Gen Genet 247:653-660; Sugita Ket al. (2000) Plant J. 22:461-469). In these systems, the recombinase (for example Cre or FLP) interacts specifically with its particular recombination sequences (34 bp lox-Sequenz and, respectively, 47 bp FRT sequence). Preference is given to the bacteriophage P1 Cre/lox and the yeast FLP/FRT systems. The FLP/FRT and cre/lox recombinase systems have already been applied in plant systems (Odell et al. (1990) Mol Gen Genet 223:369-378). Preference is given to introducing the recombinase

by means of recombinant expression starting from an expression cassette included on a DNA construct.

The activity or amount of the marker protein may also be reduced by a targeted deletion in the marker protein gene, for example by sequence-specific induction of DNA double-strand breaks at a recognition sequence for specific induction of DNA double-strand breaks in or close to the nucleic acid sequence coding for a marker protein. In its simplest embodiment (cf. Fig. 2, A and B) an enzyme is to this end introduced with the transformation construct, which generates at least one double-strand break in such a way that the resulting illegitimate recombination or deletion causes a reduction in the activity or amount of marker protein, for example by inducing a shift in the reading frame or deletion of essential sequences.

The efficiency of this approach may be increased by the sequence coding for the marker protein being flanked by sequences (A and, respectively, A') which have a sufficient length and homology to one another in order to recombine with one another as a consequence of the induced double-strand break and thus to cause, due to an intramolecular homologous recombination, a deletion of the sequence coding for the marker protein. Fig. 3 depicts diagrammatically a corresponding procedure in an exemplary embodiment of this variant.

The amount, function and/or activity of the marker protein may also be reduced by a targeted insertion of nucleic acid sequences (for example of the nucleic acid sequence to be inserted within the scope of the process of the invention) into the sequence coding for a marker protein (e.g. by means of intermolecular homologous recombination). This embodiment of the process of the invention is particularly advantageous and preferred, since, in addition to the general advantages of the process of the invention, it makes it moreover also possible to insert the nucleic acid sequence to be inserted into the plant genome in a reproducible, predictable, location-specific manner. This avoids the positional effects which otherwise occur in the course of a random, location-unspecific insertion (and which may manifest themselves, for example, in the form of different levels of expression of the transgene or in unintended inactivation of endogenous genes). Preference is given to using as an "anti-marker protein" compound in the course of this embodiment a DNA construct which comprises at least part of the sequence of a marker protein gene or neighbouring sequences and which can thus specifically recombine with said sequences in the target cell so that a deletion,

addition or substitution of at least one nucleotide alters the marker protein gene in such a way that the functionality of said marker protein gene is reduced or completely removed. The alteration may also affect the regulatory elents (e.g. the promoter) of the marker protein gene so that the coding sequence remains unaltered, but expression (transcription and/or translation) does not occur and is reduced. In conventional homologous recombination, the sequence to be inserted is flanked at its 5' and/or 3' end by further nucleic acid sequences (A' and, respectively, B') which have a sufficient length and homology to corresponding sequences of the marker protein gene (A and, respectively, B) for making homologous recombination possible. The length is usually in a range from several hundred bases to several kilobases (Thomas KR and Capecchi MR (1987) Cell 51:503; Strepp et al. (1998) Proc Natl Acad Sci USA 95(8):4368-4373). The homologous recombination is carried out by transforming the plant cell containing the recombination construct by using the process described below and selecting successfully recombined clones based on the subsequently inactivated marker protein. Although homologous recombination is a relatively rare event in plant organisms, a selection pressure may be avoided by recombination into the marker protein gene, allowing a selection of the recombined cells and sufficient efficiency of the process. Fig. 4 diagrammatically depicts a corresponding procedure in an exemplary embodiment of this variant.

In an advantageous embodiment of the invention, however, insertion into the marker protein gene is facilitated by means of further functional elements. The term is to be understood as being comprehensive and means the use of sequences or of transcripts or polypeptides derived therefrom which are capable of increasing the efficiency of the specific integration into a marker protein gene. Various processes are available to the skilled worker for this purpose. However, preference is given to implementing the insertion by inducing a sequence-specific double-strand break in or close to the marker protein gene.

In a preferred embodiment of the invention, the marker protein is inactivated (i.e. the amount, expression, activity or function is reduced) by integrating a DNA sequence into a marker protein gene, with the process preferably comprising the following steps:

i) introducing an insertion construct and at least one enzyme suitable for inducing DNA double-strand breaks at a recognition sequence for targeted induction of DNA double-strand

breaks in or close to the marker protein gene, and

ii) inducing DNA double-strand breaks at the recognition sequences for targeted induction of DNA double-strand breaks in or close to the marker protein gene, and

iii) inserting the insertion construct into the marker protein gene, with the functionality of the marker protein gene and, preferably, the functionality of the recognition sequence for targeted induction of DNA double-strand breaks is inactivated so that the enzyme suitable for induction of DNA double-strand breaks can no longer cut said recognition sequence, and

iv) selecting plants or plant cells in which the insertion construct has been inserted into the marker protein gene.

The insertion construct, preferably, comprises the nucleic acid sequence to be inserted into the genome but may also be used separately therefrom.

"Enzyme suitable for inducing DNA double-strand breaks at the recognition sequence for targeted induction of DNA double-strand breaks" ("DSBI enzyme" for "double-strand-break inducing enzyme" hereinbelow) means generally all those enzymes which are capable of generating sequence-specifically double-strand breaks in double-stranded DNA. Examples which may be mentioned but which are not limiting are:

1. Restriction endonucleases, preferably type II restriction endonucleases, particularly preferably Homing endonucleases as described in detail hereinbelow.

2. Artificial nucleases as described in detail hereinbelow, such as, for example, chimeric nucleases, mutated restriction or Homing endonucleases or RNA protein particles derived from group II mobile introns.

Both natural and artificially prepared DSBI enzymes are suitable. Preference is given to all of those DSBI enzymes whose recognition sequence is known and which can either be obtianed in the form of their proteins (for example by purification) or be expressed using their nucleic acid sequence.

Preference is given to selecting the DSBI enzyme, with the knowledge of its specific recognition sequence, in such a way that it

possesses, apart from the target recognition sequence, no further functional recognition regions in the genome of the target plant. Very particular preference is therefore given to Homing endonucleases (overview: Belfort M and Roberts RJ (1997) Nucleic Acids Res 25:3379-3388; Jasin M (1996) Trends Genet 12:224-228; Internet: http://rebase.neb.com/rebase/rebase.homing.html; Roberts RJ and Macelis D (2001) Nucl Acids Res 29: 268-269). The latter fulfill said requirement, owing to their long recognition sequences. The sequences coding for Homing endonucleases of this kind may be isolated, for example, from the Chlamydomonas chromoplast genome (Turmel M et al. (1993) J Mol Biol 232:446-467). Suitable Homing endonucleases are listed under the abovementioned internet address. Examples of Homing endonucleases which may be mentioned are those like F-SceI, F-SceII, F-SuvI, F-TevI, F-TevII, I-AmaI, I-AniI, I-CeuI, I-CeuAIIP, I-ChuI, I-CmoeI, I-CpaI, I-CpaII, I-CreI, I-CrepsbIP, I-CrepsbIIP, I-CrepsbIIIP, I-CrepsbIVP, I-CsmI, I-CvuI, I-CvuAIP, I-DdiII, I-DirI, I-DmoI, I-HspNIP, I-LlaI, I-MsoI, I-NaaI, I-NanI, I-NclIP, I-NgrIP, I-NitI, I-NjaI, I-Nsp236IP, I-PakI, I-PboIP, I-PcuIP, I-PcuAI, I-PcuVI, I-PgrIP, I-PobIP, I-PorI, I-PorIIP, I-PpbIP, I-PpoI, I-SPBetaIP, I-ScaI, I-SceI, I-SceII, I-SceIII , I-SceIV, I-SceV, I-SceVI, I-SceVII, I-SexIP, I-SneIP, I-SpomCP, I-SpomIP, I-SpomIIP, I-SquIP, I-Ssp6803I, I-SthPhiJP, I-SthPhiST3P, I-SthPhiS3bP, I-TdeIP, I-TevI, I-TevII, I-TevIII, I-UarAP, I-UarHGPA1P, I-UarHGPA13P, I-VinIP, I-ZbiIP, PI-MtuI, PI-MtuHIP, PI-MtuHIIP, PI-PfuI, PI-PfuII, PI-PkoI, PI-PkoII, PI-PspI, PI-Rma43812IP, PI-SPBetaIP, PI-SceI, PI-TfuI, PI-TfuII, PI-ThyI, PI-TliI, PI-TliII. Preference is given here to those Homing endonucleases whose gene sequences are already known, such as, for example, F-SceI, I-CeuI, I-ChuI, I-DmoI, I-CpaI, I-CpaII, I-CreI, I-CsmI, F-TevI, F-TevII, I-TevI, I-TevII, I-AniI, I-CvuI, I-LlaI, I-NanI, I-MsoI, I-NitI, I-NjaI, I-PakI, I-PorI, I-PpoI, I-ScaI, I-Ssp6803I, PI-PkoI, PI-PkoII, PI-PspI, PI-TfuI, PI-TliI.

Very particular preference is given to

- I-CeuI (Cote MJ and Turmel M (1995) Curr Genet 27:177-183.; Gauthier A et al. (1991) Curr Genet 19:43-47; Marshall (1991) Gene 104:241-245; GenBank Acc. No.: Z17234 nucleotides 5102 to 5758),

- I-ChuI (Cote V et al.(1993) Gene 129:69-76; GenBank Acc. No.: L06107, nucleotides 419 to 1075),

- I-CmoeI (Drouin M et al. (2000) Nucl Acids Res 28:4566-4572),

- I-CpaI from *Chlamydomonas pallidostigmatica* (GenBank Acc. No.: L36830, nucleotides 357 to 815; Turmel M et al. (1995) Nucleic Acids Res 23:2519-2525; Turmel, M et al. (1995) Mol Biol Evol 12:533-545)

- I-CpaII (Turmel M et al. (1995) Mol Biol Evol 12:533-545; GenBank Acc. No.: L39865, nucleotides 719 to 1423),

- I-CreI (Wang J et al. (1997) Nucleic Acids Res 25: 3767-3776; Dürrenberger, F and Rochaix JD (1991) EMBO J 10:3495-3501; GenBank Acc. No.: X01977, nucleotides 571 to 1062),

- I-CsmI (Ma DP et al. (1992) Plant Mol Biol 18:1001-1004)

- I-NanI (Elde M et al. (1999) Eur J Biochem. 259:281-288; GenBank Acc. No.: X78280, nucleotides 418 to 1155),

- I-NitI (GenBank Acc. No.: X78277, nucleotides 426 to 1163),

- I-NjaI (GenBank Acc. No.: X78279, nucleotides 416 to 1153),

- I-PpoI (Muscarella DE and Vogt VM (1989) Cell 56:443-454; Lin J and Vogt VM (1998) Mol Cell Biol 18:5809-5817; GenBank Acc. No.: M38131, nucleotides 86 to 577),

- I-PspI (GenBank Acc. No.: U00707, nucleotides 1839 to 3449),

- I-ScaI (Monteilhet C et al. (2000) Nucleic Acids Res 28: 1245-1251; GenBank Acc. No.: X95974, nucleotides 55 to 465)

- I-SceI (WO 96/14408; US 5,962,327, therein Seq ID NO: 1),

- Endo SceI (Kawasaki et al. (1991) J Biol Chem 266:5342-5347, identical to F-SceI; GenBank Acc. No.: M63839, nucleotides 159 to 1589),

- I-SceII (Sarguiel B et al. (1990) Nucleic Acids Res 18:5659-5665),

- I-SceIII (Sarguiel B et al. (1991) Mol Gen Genet. 255:340-341),

- I-Ssp6803I (GenBank Acc. No.: D64003, nucleotides 35372 to 35824),

- I-TevI (Chu et al. (1990) Proc Natl Acad Sci USA 87:3574-3578; Bell-Pedersen et al. (1990) Nucleic Acids Res18:3763-3770; GenBank Acc. No.: AF158101, nucleotides 144431 to 143694),

- I-TevII (Bell-Pedersen et al. (1990) Nucleic Acids Res 18:3763-3770; GenBank Acc. No.: AF158101, nucleotides 45612 to 44836),

- I-TevIII (Eddy et al. (1991) Genes Dev. 5:1032-1041).

Very particular preference is given to commercially available Homing endonucleases such as I-CeuI, I-SceI, I-PpoI, PI-PspI or PI-SceI. Most preference is given to I-SceI and I-PpoI. While the gene coding for I-PpoI may be utilized in its natural form, the gene coding for I-SceI possesses an editing site. Since, in contrast to yeast mitochondria, the appropriate editing is not carried out in higher plants, an artificial sequence encoding the I-SceI protein must be used for heterologous expression of this enzyme (US 5,866,361).

The enzymes may be purified from their source organisms in the manner familiar to the skilled worker and/or the nucleic acid sequence encoding said enzymes may be cloned. The sequences of various enzymes have been deposited with GenBank (see above).

Artificial DSBI enzymes which may be mentioned by way of example are chimeric nucleases which are composed of an unspecific nuclease domain and a sequence-specific DNA-binding domain (e.g. consisting of zinc fingers) (Smith J et al. (2000) Nucl Acids Res 28(17):3361-3369; Bibikova M et al. (2001) Mol Cell Biol. 21:289-297). Thus, for example, the catalytic domain of the restriction endonuclease FokI has been fused to zinc finger-binding domains, thereby defining the specificity of the endonuclease (Chandrasegaran S & Smith J (1999) Biol Chem 380:841-848; Kim YG & Chandrasegaran S (1994) Proc Natl Acad Sci USA 91:883-887; Kim YG et al. (1996) Proc Natl Acad Sci USA 93:1156-1160). The described technique has also been used previously for imparting a predefined specificity to the catalytic domain of the yeast Ho endonuclease by fusing said domain to the zinc finger domain of transcription factors (Nahon E & Raveh D (1998) Nucl Acids Res 26:1233-1239). It is possible, using suitable mutation and selection processes, to adapt existing Homing endonucleases to any de-

sired recognition sequence.

As mentioned, zinc finger proteins are particularly suitable as DNA-binding domains within chimeric nucleases. These DNA-binding zinc finger domains may be adapted to any DNA sequence. Appropriate processes for preparing corresponding zinc finger domains have been described and are known to the skilled worker (Beerli RR et al. (2000) Proc Natl Acad Sci 97(4):1495-1500; Beerli RR et al.(2000) J Biol Chem 275(42):32617-32627; Segal DJ and Barbas CF 3rd. (2000) Curr Opin Chem Biol 4(1):34-39; Kang JS and Kim JS (2000) J Biol Chem 275(12):8742-8748; Beerli RR et al. (1998) Proc Natl Acad Sci USA 95(25):14628-14633; Kim JS et al. (1997) Proc Natl Acad Sci USA 94(8):3616-3620; Klug A (1999) J Mol Biol 293(2):215-218; Tsai SY et al. (1998) Adv Drug Deliv Rev 30(1-3):23-31; Mapp AK et al. (2000) Proc Natl Acad Sci USA 97(8):3930-3935; Sharrocks AD et al. (1997) Int J Biochem Cell Biol 29(12):1371-1387; Zhang L et al. (2000) J Biol Chem 275(43):33850-33860). Processes for preparing and selecting zinc finger DNA-binding domains with high sequence specificity have been described (WO 96/06166, WO 98/53059, WO 98/53057). Fusing a DNA-binding domain obtained in this way to the catalytic domain of an endonuclease (such as, for example, the FokI or Ho endonuclease) enables chimeric nucleases to be prepared which have any desired specificity and which may be used as DSBI enzymes advantageously within the scope of the present invention.

Artificial DSBI enzymes with altered sequence specificity may also be generated by mutating already known restriction endonucleases or Homing endonucleases, using methods familiar to the skilled worker. Besides the mutagenesis of Homing endonucleases, the mutagenesis of maturases is of particular interest for the purpose of obtaining an altered substrate specificity. Maturases frequently share many features with Homing endonucleases and, if appropriate, can be converted into nucleases by carrying out few mutations. This has been shown, for example, for the maturase in the bakers' yeast bi2 intron. Only two mutations in the maturase-encoding open reading frame (ORF) sufficed to impart to this enzyme a Homing-endonuclease activity (Szczepanek & Lazowska (1996) EMBO J 15:3758-3767).

Further artificial nucleases may be generated with the aid of mobile group II introns and the proteins encoded by them, or parts of these proteins. Mobile group II introns, together with the proteins encoded by them, form RNA-protein particles which are capable of recognizing and cutting DNA in a sequence-specific manner. In this context, the sequence specificity can be adapted

to the requirements by mutating particular regions of the intron (see below) (WO 97/10362).

Preference is given to expressing the DSBI enzyme as a fusion protein with a nuclear localization sequence (NLS). This NLS sequence enables facilitated transport into the nucleus and increases the efficiency of the recombination system. Various NLS sequences are known to the skilled worker and described, inter alia, in Jicks GR and Raikhel NV (1995) Annu. Rev. Cell Biol. 11:155-188. For example, the NLS sequence of the SV40 large antigen is preferred for plant organisms. Very particular preference is given to the following NLS sequences:

NLS1: N-Pro-Lys-Thr-Lys-Arg-Lys-Val-C

NLS2: N-Pro-Lys-Lys-Lys-Arg-Lys-Val-C

Owing to the small size of many DSBI enzymes (such as, for example, the Homing endonucleases), an NLS sequence is not absolutely necessary, however. These enzymes are able to pass through the nuclear pores also without this assistance.

"Recognition sequence for targeted induction of DNA double-strand breaks" means in general those sequences which allow recognition and cleavage by the DSBI enzyme under the conditions in the eukaryotic cell or organism used in this case. In this context, mention is made, by way of example but not by limitation, in table 1 below of the recognition sequences for the particular DSBI enzymes listed.

Table 1: Recognition sequences and source organisms of DSBI enzymes ("^" indicates the cleavage site of the DSBI enzyme within a recognition sequence)

DSBI enzyme	Source organism	Recognition sequence
CRE	Bacteriophage P1	5'-AACTCTCATCGCTTCGGATAACTTCCTGTTATCCGAAACATATCACTCACTTTGGTGATTTCACCGTAACTGTCTATGATTAATG-3'
FLP	Saccharomyces cerevisiae	5'-GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTA-TAGGAACTTC-3'
R	pSR1 plasmids	5'-CGAGATCATATCACTGTGGACGTTGATGAAAGAATACGTTATTCTTTCATCAAATCGT

P-element transposase	Drosophila	5'-CTAGATGAAATAACATAAGGTGG
I-AniI	Aspergillus nidulans	5'-TTGAGGAGGTT^TCTCTGTAAATAANNNNNNNNNNNNNNN 3'-AACTCCTCCAAAGAGACATTTATTNNNNNNNNNNNNNNN^
I-DdiI	Dictyostelium discoideumAX3	5'-TTTTTTGGTCATCCAGAAGTATAT 3'-AAAAAACCAG^TAGGTCTTCATATA
I-CvuI	Chlorella vulgaris	5'-CTGGGTTCAAAACGTCGTGA^GACAGTTTGG 3'-GACCCAAGTTTTGCAG^CACTCTGTCAAACC
I-CsmI	Chlamydomonas smithii	5'-GTACTAGCATGGGGTCAAATGTCTTTCTGG
I-CmoeI	Chlamydomonas moewusii	5'-TCGTAGCAGCT^CACGGTT 3'-AGCATCG^TCGAGTGCCAA
I-CreI	Chlamydomonas reinhardtii	5'-CTGGGTTCAAAACGTCGTGA^GACAGTTTGG 3'-GACCCAAGTTTTGCAG^CACTCTGTCAAACC
I-ChuI	Chlamydomonas humicola	5'-GAAGGTTTGGCACCTCG^ATGTCGGCTCATC 3'-CTTCCAAACCGTG^GAGCTACAGCCGAGTAG
I-CpaI	Chlamydomonas pallidostigmatica	5'-CGATCCTAAGGTAGCGAA^ATTCA 3'-GCTAGGATTCCATC^GCTTTAAGT
I-CpaII	Chlamydomonas pallidostigmatica	5'-CCCGGCTAACTC^TGTGCCAG 3'-GGGCCGAT^TGAGACACGGTC
I-CeuI	Chlamydomonas eugametos	5'-CGTAACTATAACGGTCCTAA^GGTAGCGAA 3'-GCATTGATATTGCCAG^GATTCCATCGCTT
I-DmoI	Desulfurococcus mobilis	5'-ATGCCTTGCCGGGTAA^GTTCCGGCGCGCAT 3'-TACGGAACGGCC^CATTCAAGGCCGCGCGTA
I-SceI	S.cerevisiae	5'-AGTTACGCTAGGGATAA^CAGGGTAATATAG 3'-TCAATGCGATCCC^TATTGTCCCATTATATC 5'-TAGGGATAA^CAGGGTAAT 3'-ATCCC^TATTGTCCCATTA ("Core" sequence)
I-SceII	S.cerevisiae	5'-TTTTGATTCTTTGGTCACCC^TGAAGTATA 3'-AAAACTAAGAAACCAG^TGGGACTTCATAT
I-SceIII	S.cerevisiae	5'-ATTGGAGGTTTTGGTAAC^TATTTATTACC 3'-TAACCTCCAAAACC^ATTGATAAATAATGG
I-SceIV	S.cerevisiae	5'-TCTTTTCTCTTGATTA^GCCCTAATCTACG 3'-AGAAAAGAGAAC^TAATCGGGATTAGATGC

I-SceV	S.cerevisiae	5'-AATAATTTTCT^TCTTAGTAATGCC 3'-TTATTAAAAGAAGAATCATTA^CGG
I-SceVI	S.cerevisiae	5'-GTTATTTAATG^TTTTAGTAGTTGG 3'-CAATAAATTACAAAATCATCA^ACC
I-SceVII	S.cerevisiae	5'-TGTCACATTGAGGTGCACTAGTTATTAC
PI-SceI	S.cerevisiae	5'-ATCTATGTCGGGTGC^GGAGAAAGAGGTAAT 3'-TAGATACAGCC^CACGCCTCTTTCTCCATTA
F-SceI	S.cerevisiae	5'-GATGCTGTAGGC^ATAGGCTTGGTT 3'-CTACGACA^TCCGTATCCGAACCAA
F-SceII	S.cerevisiae	5'-CTTTCCGCAACA^GTAAAATT 3'-GAAAGGCG^TTGTCATTTTAA
I-HmuI	Bacillus subtilis bacteriophage SPO1	5'-AGTAATGAGCCTAACGCTCAGCAA 3'-TCATTACTCGGATTGC^GAGTCGTT
I-HmuII	Bacillus subtilis bacteriophage SP82	5'-AGTAATGAGCCTAACGCTCAACAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
I-LlaI	Lactococcus lactis	5'-CACATCCATAAC^CATATCATTTTT 3'-GTGTAGGTATTGGTATAGTAA^AAA
I-MsoI	Monomastix species	5'-CTGGGTTCAAAACGTCGTGA^GACAGTTTGG 3'-GACCCAAGTTTTGCAG^CACTCTGTCAAACC
I-NanI	Naegleria andersoni	5'-AAGTCTGGTGCCA^GCACCCGC 3'-TTCAGACC^ACGGTCGTGGGCG
I-NitI	Naegleria italica	5'-AAGTCTGGTGCCA^GCACCCGC 3'-TTCAGACC^ACGGTCGTGGGCG
I-NjaI	Naegleria jamiesoni	5'-AAGTCTGGTGCCA^GCACCCGC 3'-TTCAGACC^ACGGTCGTGGGCG
I-PakI	Pseudendoclonium akinetum	5'-CTGGGTTCAAAACGTCGTGA^GACAGTTTGG 3'-GACCCAAGTTTTGCAG^CACTCTGTCAAACC
I-PorI	Pyrobaculum organotrophum	5'-GCGAGCCCGTAAGGGT^GTGTACGGG 3'-CGCTCGGGCATT^CCCACACATGCCC
I-PpoI	Physarum polycephalum	5'-TAACTATGACTCTCTTAA^GGTAGCCAAAT 3'-ATTGATACTGAGAG^AATTCCATCGGTTTA
I-ScaI	Saccharomyces capensis	5'-TGTCACATTGAGGTGCACT^AGTTATTAC 3'-ACAGTGTAACTCCAC^GTGATCAATAATG

I-Ssp6803I	Synechocystis species	5'-GTCGGGCT^CATAACCCGAA 3'-CAGCCCGAGTA^TTGGGCTT
PI-PfuI	Pyrococcus furiosus Vc1	5'-GAAGATGGGAGGAGGG^ACCGGACTCAACTT 3'-CTTCTACCCTCC^TCCCTGGCCTGAGTTGAA
PI-PfuII	Pyrococcus furiosus Vc1	5'-ACGAATCCATGTGGAGA^AGAGCCTCTATA 3'-TGCTTAGGTACAC^CTCTTCTCGGAGATAT
PI-PkoI	Pyrococcus kodakaraensis KOD1	5'-GATTTTAGAT^CCCTGTACC 3'-CTAAAA^TCTAGGGACATGG
PI-PkoII	Pyrococcus kodakaraensis KOD1	5'-CAGTACTACG^GTTAC 3'-GTCATG^ATGCCAATG
PI-PspI	Pyrococcus sp.	5'-AAAATCCTGGCAAACAGCTATTAT^GGGTAT 3'-TTTTAGGACCGTTTGTCGAT^AATACCCATA
PI-TfuI	Thermococcus fumicolans ST557	5'-TAGATTTTAGGT^CGCTATATCCTTCC 3'-ATCTAAAA^TCCAGCGATATAGGAAGG
PI-TfuII	Thermococcus fumicolans ST557	5'-TAYGCNGAYACN^GACGGYTTYT 3'-ATRCGNCT^RTGNCTGCCRAARA
PI-ThyI	Thermococcus hydrothermal-is	5'-TAYGCNGAYACN^GACGGYTTYT 3'-ATRCGNCT^RTGNCTGCCRAARA
PI-TliI	Thermococcus litoralis	5'-TAYGCNGAYACNGACGG^YTTYT 3'-ATRCGNCTRTGNC^TGCCRAARA
PI-TliII	Thermococcus litoralis	5'-AAATTGCTTGCAAACAGCTATTACGGCTAT
I-TevI	Bacteriophage T4	5'-AGTGGTATCAAC^GCTCAGTAGATG 3'-TCACCATAGT^TGCGAGTCATCTAC
I-TevII	Bacteriophage T4	5'-GCTTATGAGTATGAAGTGAACACGT^TATTC 3'-CGAATACTCATACTTCACTTGTG^CAATAAG
F-TevI	Bacteriophage T4	5'-GAAACACAAGA^AATGTTTAGTAAANNNNNNNNNNNNNN 3'-CTTTGTGTTCTTTACAAATCATTTNNNNNNNNNNNNNN^
F-TevII	Bacteriophage T4	5'-TTTAATCCTCGCTTC^AGATATGGCAACTG 3'-AAATTAGGAGCGA^AGTCTATACCGTTGAC

Relatively small deviations (degenerations) of the recognition sequence which nevertheless make possible recognition and cleavage by the particular DSBI enzyme are also included here. Such

deviations, also in connection with different basic conditions such as, for example, calcium or magnesium concentration, have been described (Argast GM et al. (1998) J Mol Biol 280:345-353). Core sequences of these recognition sequences are also included. It is known that the inner portions of the recognition sequences also suffice for an induced double-strand break and that the outer portions are not necessarily relevant but may contribute to determining the cleavage efficiency. Thus, for example, an 18bp core sequence can be defined for I-SceI.

Said DSBI recognition sequences may be localized in various positions in or close to a marker protein gene and, for example when the marker protein used is a transgene, may already be incorporated when constructing the marker protein expression cassette. Various possible localizations are illustrated by way of example in Figs. 2-A, 2-B, 3 and 5 and in the descriptions thereof.

In a further advantageous embodiment, the insertion sequence comprises at least one homology sequence A which has a sufficient length and a sufficient homology to a sequence A' in the marker protein gene in order to ensure homologous recombination between A and A'. The insertion sequence is preferably flanked by two sequences A and B which have a sufficient length and a sufficient homology to a sequence A' and, respectively, B' in the marker protein gene in order to ensure homologous recombination between A and A' and, respectively, B and B'.

"Sufficient length" means, with respect to the homology sequences A, A' and B, B', preferably sequences with a length of at least 100 base pairs, preferably at least 250 base pairs, particularly preferably at least 500 base pairs, very particularly preferably at least 1000 base pairs, most preferably of at least 2500 base pairs.

"Sufficient homology" means, with respect to the homology sequences, preferably sequences whose homology to one another is at least 70%, preferably 80%, preferentially at least 90%, particularly preferably at least 95%, very particularly preferably at least 99%, most preferably 100%, over a length of at least 20 base pairs, preferably at least 50 base pairs, particularly preferably at least 100 base pairs, very particularly preferably at least 250 base pairs, most preferably at least 500 base pairs.

Homology between two nucleic acids means the identity of the nucleic acid sequence over in each case the entire sequence length, which identity is calculated by way of comparison with the aid of

the GAP program algorithm (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 12	Length Weight: 4
Average Match: 2,912	Average Mismatch:-2,003

In a further preferred embodiment, the recombination efficiency is increased by a combination with processes which promote homologous recombination. Such systems have been described and comprise, by way of example, expression of proteins such as RecA or treatment with PARP inhibitors. It has been demonstrated that the intrachromosomal homologous recombination in tobacco plants can be increased by using PARP inhibitors (Puchta H et al. (1995) Plant J 7:203-210). The use of these inhibitors can further increase the rate of homologous recombination in the recombinant constructs, after inducing the sequence-specific DNA double-strand break, and thus the efficiency of the deletion of the transgene sequences. Various PARP inhibitors may be used here. Preference is given to including inhibitors such as 3-amino benzamide, 8-hydroxy-2-methylquinazolin-4-one (NU1025), 1,11b-dihydro-[2H]benzopyrano[4,3,2-de]isoquinolin-3-one (GPI 6150), 5-aminoisoquinolinone, 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone or the substances described in WO 00/26192, WO 00/29384, WO 00/32579, WO 00/64878, WO 00/68206, WO 00/67734, WO 01/23386 and WO 01/23390.

Further suitable methods are the introduction of nonsense mutations into endogenous marker protein genes, for example by means of introducing RNA/DNA oligonucleotides into the plant (Zhu et al. (2000) Nat Biotechnol 18(5):555-558). Point mutations may also be generated by means of DNA-RNA hybrids which are also known as "chimeraplasty" (Cole-Strauss et al. (1999) Nucl Acids Res 27(5):1323-1330; Kmiec (1999) Gene therapy American Scientist 87(3):240-247).

The methods of dsRNAi, cosuppression by means of sense RNA and VIGS (virus induced gene silencing) are also referred to as post-transcriptional gene silencing (PTGS). PTGS processes are particularly advantageous because the demands on the homology between the marker protein gene to be reduced and the transgenically expressed sense or dsRNA nucleic acid sequence are lower than, for example, in the case of a traditional antisense approach. Thus it is possible, using the marker protein nucleic acid sequences from one species, to effectively reduce also expression of homologous

marker protein proteins in other species, without it being absolutely necessary to isolate and to elucidate the structure of the marker protein homologues occurring there. Considerably less labor is therefore required.

"Introduction" comprises within the scope of the invention any processes which are suitable for introducing an "anti-marker protein" compound, directly or indirectly, into a plant or a cell, compartment, tissue, organ or seeds of said plant or generating said compound there. The introduction may result in a transient presence of an "anti-marker protein" compound (for example a dsRNA or a recombinase) or else in a permanent (stable) presence.

According to the different nature of the approaches described above, the "anti-marker protein" compound may exert its function directly (for example by way of insertion into an endogenous marker protein gene). However, said function may also be exerted indirectly after transcription into an RNA (for example in antisense approaches) or after transcription and translation into a protein (for example in the case of recombinases or DSBI enzymes). The invention comprises both directly and indirectly acting "anti-marker protein" compounds.

Introducing comprises, for example, processes such as transfection, transduction or transformation.

"Anti-marker protein" compounds thus comprises, for example, also expression cassettes capable of implementing expression (i.e. transcription and, if appropriate, translation) of, for example, an MP-dsRNA, an MP-antisenseRNA, a sequence-specific recombinase or a DSBI enzyme in a plant cell.

"Expression cassette" means within the scope of the present invention generally those constructions in which a nucleic acid sequence to be expressed is functionally linked to at least one genetic control sequence, preferably a promoter sequence. Expression cassettes preferably consist of double-stranded DNA and may have a linear or circular structure.

A functional linkage means, for example, the sequential arrangement of a promoter with a nucleic acid sequence to be transcribed (for example coding for an MP-dsRNA or a DSBI enzyme) and, if appropriate, further regulatory elements such as, for example, a terminator and/or polyadenylation signals in such a way that each of the regulatory elements can fulfill its function during transcription of the nucleic acid sequence, depending on the arrange-

ment of the nucleic acid sequences. In this context, function can mean, for example, the control of expression, i.e. transcription and/or translation, of the nucleic acid sequence (e.g. coding for an MP-dsRNA or a DSBI enzyme). In this context, control comprises, for example, initiating, increasing, controlling or suppressing the expression, i.e. transcription and, if appropriate, translation. This does not necessarily require a direct linkage in the chemical sense. Genetic control sequences such as, for example, enhancer sequences, may exert their function on the target sequence also from positions further afar or even from different DNA molecules. Preference is given to arrangements in which the nucleic acid sequence to be transcribed is positioned downstream of the sequence acting as promoter so that both sequences are covalently connected to one another. The distance between the promoter sequence and the nucleic acid sequence to be expressed transgenically is here preferably less than 200 base pairs, particularly preferably less than 100 base pairs, very particularly preferably less than 50 base pairs.

The skilled worker knows various ways of obtaining any of the expression cassettes of the invention. An expression cassette of the invention is prepared, for example, preferably by direct fusion of a nucleic acid sequence acting as promoter to a nucleotide sequence to be expressed (e.g. coding for an MP-dsRNA or a DSBI enzyme). A functional linkage may be produced by means of common recombination and cloning techniques, as are described, for example, in Maniatis T, Fritsch EF and Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and in Silhavy TJ et al. (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and in Ausubel FM et al.(1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience.

The expression cassettes of the invention preferably comprise a promoter 5' upstream of the particular nucleic acid sequence to be expressed transgenically and a terminator sequence as an additional genetic control sequence 3' downstream and also, if appropriate, further customary regulatory elements, in each case functionally linked to the nucleic acid sequence to be expressed transgenically.

The term "genetic control sequences" is to be understood broadly and means all those sequences which have an influence on the making or function of the expression cassette of the invention. For example, genetic control sequences ensure transcription and, if

appropriate, translation in prokaryotic or eukaryotic organisms. Genetic control sequences are described, for example, in "Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990)" or "Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press, Boca Raton, Florida, eds.:Glick and Thompson, Chapter 7, 89-108" and in the references quoted there.

Genetic control sequences comprise, in particular in plants, functional promoters. Preferred promoters suitable for the expression cassettes are in principle any promoters capable of controlling expression of genes, in particular foreign genes, in plants.

Plant-specific promoters or promoters functional in plants or in a plant cell means in principle any promoter capable of controlling expression of genes, in particular foreign genes, in at least one plant or one part, cell, tissue, culture of a plant. In this context, expression may be, for example, constitutive, inducible or development-dependent. Preference is given to:

a) Constitutive promoters

"Constitutive" promoters means those promoters which ensure expression in numerous, preferably all, tissues over a relatively large period of plant development, preferably at all points in time of plant development (Benfey et al.(1989) EMBO J 8:2195-2202). Preference is given in particular to using a plant promoter or a promoter which is derived from a plant virus. Particular preference is given to the promoter of the 35S transcript of the CaMV cauliflower mosaic virus (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221- 228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202) and also to the promoter of the Arabidopsis thaliana nitrilase-1 gene (GenBank Acc. No.: Y07648, nucleotides 2456 (alternatively 2861) to 4308 or alternatively 4340 or 4344. (e.g. bp 2456 to 4340).

Another suitable constitutive promoter is the rubisco small subunit (SSU) promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No.: X03677), the promoter of the Agrobacterium nopaline synthase, the TR dual promoter, the Agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubi-

quitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker.

b) Tissue-specific promoters

Preference is given to promoters with specificities for the anthers, ovaries, flowers, leaves, stems, roots or seeds.

Seed-specific promoters comprise, for example, the promoter of phaseolin (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), of the 2S albumin (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), of legumin (Shirsat A et al. (1989) Mol Gen Genet 215(2): 326-331), of USP (unknown seed protein; Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), of napin (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), of the sucrose-binding protein (WO 00/26388), of legumin B4 (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Baeumlein et al. (1992) Plant Journal 2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), of oleosin (WO 98/45461) or of Bce4 (WO 91/13980). Further suitable seed-specific promoters are those of the genes coding for the high molecular weight glutenin (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase) or starch synthase. Preference is further given to promoters which allow seed-specific expression in monocotyledones such as corn, barley, wheat, rye, rice, etc. promoters which may be employed advantageously are the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) and the promoters described in WO 99/16890 (hordein, glutelin, oryzin, prolamin, gliadin, zein, kasirin or secalin promoters). Further seed-specific promoters are described in WO 89/03887.

Tuber-, storage-root- or root-specific promoters comprise, for example, the class I patatin promoter (B33) or the promoter of the potato cathepsin D inhibitor.

Leaf-specific promoters comprise, for example, the promoter of the potato cytosolic FBPase (WO 97/05900), the

SSU promoter (small subunit) of rubisco (ribulose-1,5-bisphosphate carboxylase) or the potato ST-LSI promoter (Stockhaus et al. (1989) EMBO J 8:2445-2451).

Flower-specific promoters comprise, for example, the phytoene synthase promoter (WO 92/16635) or the promoter of the P-rr gene (WO 98/22593).

- Anther-specific promoters comprise, for example, the 5126 promoter (US 5,689,049, US 5,689,051), the glob-1 promoter and the γ-zein promoter.

c) Chemically inducible promoters

Chemically inducible promoters allow expression control as a function of an exogenous stimulus (review article: Gatz et al. (1997) Ann Rev Plant Physiol Plant Mol Biol 48:89-108). Examples which may be mentioned are: the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), a salicylic acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP-A 0 388 186), a tetracycline-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic acid-inducible promoter (EP 0 335 528) and an ethanol- or cyclohexanone-inducible promoter (WO 93/21334). Also suitable is the promoter of the glutathione S-transferase isoform II gene (GST-II-27), which may be activated by exogenously applied safeners such as, for example, N,N-diallyl-2,2-dichloroacetamide (WO 93/01294) and which is functional in numerous tissues of both monocotyledones and dicotyledones.

Particular preference is given to constitutive or inducible promoters.

Preference is further given to plastid-specific promoters for targeted expression in the plastids. Suitable promoters are described, for example, in WO 98/55595 or WO 97/06250. promoters which may be mentioned here are the rpo B promoter element, the atoB promoter element, the clpP promoter element (see also WO 99/46394) and the 16SrDNA promoter element. Viral promoters are also suitable (WO 95/16783).

Targeted expression in plastids may also be achieved by using, for example, a bacterial or bacteriophage promoter, introducing the resulting expression cassette into the plastid DNA and then expressing expression by means of a fusion protein of a bacterial or bacteriophage polymerase and a plastid transit peptide. US

5,925,806 describes an appropriate process.

Genetic control sequences further comprise also the 5'-untranslated regions, introns or noncoding 3' region of genes, such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (general overview: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). These sequences have been shown to be able to play a significant functions in the regulation of gene expression. Thus it has been demonstrated that 5'-untranslated sequences may increase transient expression of heterologous genes. They may further promote tissue specificity (Rouster J et al.(1998) Plant J. 15:435-440). As an example of translation enhancers, mention may be made of the 5' leader sequence of the tobacco mosaic virus (Gallie et al. (1987) Nucl Acids Res 15:8693-8711).

Polyadenylation signals suitable as control sequences are in particular polyadenylation signals of plant genes and also *Agrobacterium tumefaciens* T-DNA polyadenylation signals. Examples of particularly suitable terminator sequences are the OCS (octopine synthase) terminator and the NOS (nopaline synthase) terminator (Depicker A et al (1982) J Mol Appl Genet 1:561-573) and also the terminators of soybean actin, RUBISCO or alpha-amylase from wheat (Baulcombe DC et al (1987) Mol Gen Genet 209:33-40).

Advantageously, the expression cassette may contain one or more "enhancer sequences" functionally linked to the promoter, which make increased transgenic expression of the nucleic acid sequence possible.

Genetic control sequences further means sequences coding for fusion proteins consisting of a signal peptide sequence. The expression of a target gene is possible in any desired cell compartment, such as, for example, the endomembrane system, the vacuole and the chloroplasts. Desired glycosylation reactions, in particular foldings, and the like are possible by utilizing the secretory pathway. Secretion of the target protein to the cell surface or secretion into the culture medium, for example when using suspension-cultured cells or protoplasts, is also possible. The target sequences required for this may both be taken into account in individual vector variations and be introduced into the vector together with the target gene to be cloned by using a suitable cloning strategy. Target sequences which may be used are both endogenous, if present, and heterologous sequences. Additional heterologous sequences which are preferred for functional linkage but not limited thereto are further targeting sequences

for ensuring subcellular localization in the apoplast, in the vacuole, in plastids, in the mitochrondrion, in the endoplasmic reticulum (ER), in the nucleus, in elaioplasts or other compartments; and also translation enhancers such as the 5' leader sequence from tobacco mosaic virus (Gallie et al. (1987) Nucl Acids Res 15: 8693-8711) and the like. The process of transporting proteins which are per se not located in the plastids specifically into said plastids has been described (Klosgen RB and Weil JH (1991) Mol Gen Genet 225(2):297-304; Van Breusegem F et al. (1998) Plant Mol Biol 38(3):491-496).

Control sequences are furthermore understood to be those which make possible a homologous recombination or insertion into the genome of a host organism or allow the removal from the genome. Methods such as the cre/lox technique allow the expression cassette to be removed tissue-specifically, possibly inducibly from the genome of the host organism (Sauer B. Methods. 1998; 14(4):381-92). Here, particular flanking sequences are attached to the target gene (lox sequences), which make subsequent removal by means of the cre recombinase possible.

Preferably, the expression cassette, consisting of a linkage of the promoter to the nucleic acid sequence to be transcribed, may have been integrated into a vector and may be transferred into the plant cell or organism, for example, by transformation, according to any of the processes described below.

"Transgenic" means preferably, for example with respect to a transgenic expression cassette, a transgenic expression vector, a transgenic organism or to processes for transgenic expression of nucleic acids, all constructions brought about by genetic engineering methods or processes using said constructions, in which either

a) the nucleic acid sequence to be expressed, or

b) the promoter functionally linked to the nucleic acid sequence to be expressed according to a), or

c) (a) and (b)

are not located in their natural, genetic environment (i.e. at their natural chromosomal locus) or have been modified by genetic engineering methods, the modification possibly being, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment

means the natural chromosomal locus in the source organism or the presence in a genomic library.

"Transgenic" means, with respect to expression ("transgenic expression"), preferably all expressions achieved using a transgenic expression cassette, transgenic expression vector or transgenic organism, according to the definitions indicated above.

The DNA constructs employed within the scope of the process of the invention and the vectors derived therefrom may contain further functional elements. The term functional element is to be understood broadly and means all of those elements which influence the preparation, propagation or function of the DNA constructs or of vectors or organisms derived therefrom. Examples which may be mentioned without being limited thereto are:

1. Selection markers

Selection markers comprise, for example, those nucleic acid or protein sequences whose expression gives to a cell, tissue or organism an advantage (positive selection marker) or disadvantage (negative selection marker) over cells which do not express said nucleic acid or protein. Positive selection markers act, for example, by detoxifying a substance acting on the cell in an inhibitory manner (e.g. resistance to antibiotics/herbicides) or by forming a substance which enables the plant to regenerate better or grow more under the chosen conditions (for example nutritive markers, hormone-producing markers such as ipt; see below). Another type of positive selection mareker comprises mutated proteins or RNAs which are not sensitive to a selective agent (e.g. 16S rRNA mutants which are insensitive to spectinomycin). Negative selection markers act, for example, by catalyzing the formation of a toxic substance in the transformed cells (e.g. the codA gene).

1.1 Positive selection markers:

In order to further increase the efficiency, the DNA constructs may comprise additional positive selection markers. In a preferred embodiment, the process of the invention may thus be carried out in the form of a dual selection in which a sequence coding for a resistance to at least one toxin, antibiotic or herbicide is introduced together with the nucleic acid sequence to be inserted and selection is carried out additionally by using the toxin, antibiotic or herbicide.

Appropriate proteins and sequences of positive selection markers and also selection processes are familiar to the skilled worker. The selection marker imparts to the successfully transformed cells a resistance to a biocide (e.g. a herbicide such as phosphinothricin, glyphosate or bromoxynil), a metabolism inhibitor such as 2-deoxyglucose 6-phosphate (WO 98/45456) or an antibiotic such as, for example, tetracycline, ampicillin, kanamycin, G 418, neomycin, bleomycin or hygromycin. Selection markers which may be mentioned by way of example are:

- phosphinothricin acetyltransferases (PAT) which acetylate the free amino group of the glutamine synthase inhibitor phosphinothricin (PPT) and thus detoxify PPT (de Block et al. (1987) EMBO J 6:2513-2518) (also referred to as Bialophos® resistance gene (bar)). Corresponding sequences are known to the skilled worker (from Streptomyces hygroscopicus GenBank Acc. No.: X17220 and X05822, from Streptomyces viridochromogenes GenBank Acc. No.: M 22827 and X65195; US 5,489,520). Furthermore, synthetic genes have been described for expression in plastids. A synthetic PAT gene is described in Becker et al. (1994) Plant J 5:299-307. The genes impart a resistance to the herbicide Bialaphos or glufosinate and are frequently used markers in transgenic plants (Vickers JE et al. (1996) Plant Mol Miol Reporter 14:363–368; Thompson CJ et al. (1987) EMBO J 6:2519–2523).

- 5-enolpyruvylshikimate 3-phosphate synthases (EPSPS) which impart a resistance to glyphosate (N-(phosphonomethyl) glycine). The molecular target of the unselective herbicide glyphosate is 5-enolpyruvyl-3-phosphoshikimate synthase (EPSPS). This enzyme has a key function in the biosynthesis of aromatic amino acids in microbes and plants but not in mammals (Steinrucken HC et al. (1980) Biochem Biophys Res Commun 94:1207–1212; Levin JG and. Sprinson DB (1964) J Biol Chem 239:1142–1150; Cole DJ (1985) Mode of action of glyphosate a literature analysis, p. 48–74. In: Grossbard E and Atkinson D (eds.). The herbicide glyphosate. Buttersworths, Boston.). Preference is given to using glyphosate-tolerant EPSPS variants as selection markers (Padgette SR et al. (1996). New weed control opportunities: development of soybeans with a Roundup Ready™ gene. In: Herbicide Resistant Crops (Duke, S.O., ed.), pp. 53-84. CRC Press, Boca Raton, FL; Saroha MK and Malik VS (1998) J Plant Biochemistry and Biotechnology 7:65–72). The EPSPS gene of Agrobacterium sp. strain CP4 has a natural tolerance for glyphosate, which can be transferred to appropriate transgenic plants. The CP4 EPSPS gene was cloned from Agrobacterium sp. strain CP4 (Pad-

gette SR et al. (1995)Crop Science 35(5):1451–1461). Sequences of EPSPS enzymes which are glyphosate-tolerant have been described (inter alia in US 5,510,471; US 5,776,760; US 5,864,425; US 5,633,435; US 5,627;061; US 5,463,175; EP 0 218 571). Further sequences are described under GenBank Acc. No: X63374 or M10947.

- Glyphosat®-degrading enzymes (gox gene; glyphosate oxidoreductase). GOX (for example Achromobacter sp. glyphosate oxidoreductase) catalyzes the cleavage of a C-N bond in glyphosate which is thus converted to aminomethylphosphonic acid (AMPA) and glyoxylate. GOX can thereby impart a resistance to glyphosate (Padgette SR et al. (1996) J Nutr 126(3):702-16; Shah D et al. (1986) Science 233:478-481).

- The deh gene encodes a dehalogenase which inactivates Dalapon® (GenBank Acc. No.: AX022822, AX022820 and WO 99/27116)

- The bxn genes encode bromoxynil-degrading nitrilase enzymes (Genbank Acc. No: E01313 and J03196).

- Neomycin phosphotransferases impart a resistance to antibiotics (aminoglycosides) such as neomycin, G418, hygromycin, paromomycin or kanamycin by reducing the inhibiting action of said antibiotics by means of a phosphorylation reaction. Particular preference is given to the nptII gene. Sequences can be obtained from GenBank (AF080390; AF080389). Moreover, the gene is already part of numerous expression vectors and can be isolated therefrom using processes familiar to the skilled worker (AF234316; AF234315; AF234314). The NPTII gene encodes an aminoglycoside 3'-O-phosphotransferase from E.coli, Tn5 (GenBank Acc. No: U00004 position 1401-2300; Beck et al. (1982) Gene 19 327-336).

- The DOG^{R}1 gene was isolated from the yeast Saccharomyces cerevisiae (EP-A 0 807 836) and encodes a 2-deoxyglucose 6-phosphate phosphatase which imparts a resistance to 2-DOG (Randez-Gil et al. (1995) Yeast 11:1233-1240; Sanz et al. (1994) Yeast 10:1195-1202, GenBank Acc. No.: NC001140; position 194799-194056).

- Acetolactate synthases which impart a resistance to imidazolinone/sulfonylurea herbicides (GenBank Acc. No.: X51514; Sathasivan K et al. (1990) Nucleic Acids Res. 18(8):2188); AB049823; AF094326; X07645; X07644; A19547; A19546; A19545;

I05376; I05373; AL133315)

- Hygromycin phosphotransferases (e.g. GenBank Acc. No.: X74325) which impart a resistance to the antibiotic hygromycin. The gene is part of numerous expression vectors and may be isolated therefrom using processes familiar to the skilled worker (such as, for example, polymerase chain reaction) (GenBank Acc. No.: AF294981; AF234301; AF234300; AF234299; AF234298; AF354046; AF354045).

- Genes of resistance to

  a) Chloramphenicol (chloramphenicol acetyltransferase),

  b) tetracycline (inter alia GenBank Acc. No.: X65876; X51366). Moreover, the gene is already part of numerous expression vectors and may be isolated therefrom using processes familiar to the skilled worker (such as, for example, polymerase chain reaction)

  c) Streptomycin (inter alia GenBank Acc. No.: AJ278607).

  d) Zeocin, the corresponding resistance gene is part of numerous cloning vectors (e.g. GenBank Acc. No.: L36849) and may be isolated therefrom using processes familiar to the skilled worker (such as, for example, polymerase chain reaction).

  e) Ampicillin (ß-lactamase gene; Datta N, Richmond MH (1966) Biochem J 98(1):204-9; Heffron F et al (1975) J. Bacteriol 122: 250–256; Bolivar F et al. (1977) Gene 2:95–114). The sequence is part of numerous cloning vectors and may be isolated therefrom using processes familiar to the skilled worker (such as, for example, polymerase chain reaction).

Genes such as isopentenyl transferase from Agrobacterium tumefaciens (strain:PO22) (Genbank Acc. No.: AB025109) may also be used as selection markers. The ipt gene is a key enzyme of cytokinin biosynthesis. Its overexpression facilitates the regeneration of plants (e.g. selection on cytokinin-free medium). The process for utilizing the ipt gene has been described (Ebinuma H et al. (2000) Proc Natl Acad Sci USA 94:2117-2121; Ebinuma H et al. (2000) Selection of Marker-free transgenic plants using the oncogenes (*ipt*, *rol* A, B, C) of Agrobacterium as selectable markers, In Molecular Biology of Woody Plants. Kluwer Academic Publish-

ers).

Various other positive selection markers which impart to the transformed plants a growth advantage over untransformed plants and also processes for their use are described, inter alia, in EP-A 0 601 092. Examples which may be mentioned are β-glucuronidase (in connection with cytokinin glucuronide, for example), mannose 6-phosphate isomerase (in connection with mannose), UDP-galactose 4-epimerase (in connection with galactose, for example).

For a selection marker functional in plastids, particular preference is given to those which impart a resistance to spectinomycin, streptomycin, kanamycin, lincomycin, gentamycin, hygromycin, methotrexat, bleomycin, phleomycin, blasticidin, sulfonamide, phosphinothricin, chlorsulfuron, bromoxymil, glyphosate, 2,4-datrazine, 4-methyltryptophan, nitrate, S-aminoethyl-L-cysteine, lysine/threonine, aminoethyl-cysteine or betainealdehyde. Particular preference is given to the genes aadA, nptII, BADH, FLARE-S (a fusion of aadA and GFP, described in Khan MS & Maliga P (1999) Nature Biotech 17:910-915). Especially suitable is the aadA gene (Svab Z and Maliga P (1993) Proc Natl Acad Sci USA 90:913-917). Modified 16S rDNA and also betainealdehyde dehydrogenase (BADH) from spinach have also been described (Daniell H et al. (2001) Trends Plant Science 6:237-239; Daniell H et al. (2001) Curr Genet 39:109-116; WO 01/64023; WO 01/64024; WO 01/64850). Lethal agents such as, for example, glyphosate may also be utilized in connection with correspondingly detoxifying or resistance enzymes (WO 01/81605).

The concentrations of the antibiotics, herbicides, biocides or toxins, which are used in each case for selection, must be adapted to the particular test conditions or organisms. Examples which may be mentioned for plants are kanamycin (Km) 50 mg/L, hygromycin B 40 mg/L, phosphinothricin (Ppt) 6 mg/L, spectinomycin (Spec) 500 mg/L.

2. Reporter genes

Reporter genes code for readily quantifiable proteins and thus ensure, via intrinsic color or enzyme activity, an evaluation of the transformation efficiency and of the location or time of expression. In this context, very particular preference is given to genes coding for reporter proteins (see also Schenborn E, Groskreutz D (1999) Mol Biotechnol 13(1):29-44) such as

- green fluorescence protein (GFP) (Chui WL et al. (1996) Curr Biol 6:325-330; Leffel SM et al. (1997) Biotechniques 23(5):912-8; Sheen et al. (1995) Plant J 8(5):777-784; Haseloff et al. (1997) Proc Natl Acad Sci USA 94(6): 2122-2127; Reichel et al. (1996) Proc Natl Acad Sci USA 93(12):5888-5893; Tian et al. (1997) Plant Cell Rep 16:267-271; WO 97/41228)

- chloramphenicol transferase

- luciferase (Millar et al. (1992) Plant Mol Biol Rep 10: 324-414; Ow et al. (1986) Science 234:856-859); allows bioluminescence detection

- β-galactosidase (encodes an enzyme for which various chromogenic substrates are available)

- ß-glucuronidase (GUS) (Jefferson et al. (1987) EMBO J 6: 3901-3907) or the uidA gene (encode enzymes for which various chromogenic substrates are available)

- R-locus gene product which regulates production of anthocyanin pigments (red color) in plant tissue and thus makes possible a direct analysis of the promoter activity without addition of additional auxiliary substances or chromogenic substrates (Dellaporta et al. (1988) In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium, 11:263-282)

- tyrosinase (Katz et al.(1983) J Gen Microbiol 129:2703-2714), enzyme which oxidizes tyrosine to give DOPA and dopaquinone which consequently form the readily detectable melanine.

- aequorin (Prasher et al.(1985) Biochem Biophys Res Commun 126(3):1259-1268), may be used in calcium-sensitive bioluminescence detection.

3. Origins of replication which ensure propagation of the expression cassettes or vectors of the invention, for example in E. coli. Examples which may be mentioned are ORI (origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

4. Elements, for example border sequences, which enable agrobacteria-mediated transfer into plant cells for transfer and integration into the plant genome, such as, for example, the right or left border of T-DNA or the vir region.

5. Multiple cloning regions (MCS) allow and facilitate the insertion of one or more nucleic acid sequences.

Nucleic acid sequences (e.g. expression cassettes) may be introduced into a plant organism or cells, tissues, organs, parts or seeds thereof by advantageously using vectors which contain said sequences. Vectors may be, by way of example, plasmids, cosmids, phages, viruses or else agrobacteria. The sequences may be inserted into the vector (preferably a plasmid vector) via suitable restriction cleavage sites. The resulting vector may first be introduced into E. coli and amplified. Correctly transformed E. coli are selected, grown and the recombinant vector is obtained using methods familiar to the skilled worker. Restriction analysis and sequencing may serve to check the cloning step. Preference is given to those vectors which make possible a stable integration into the host genome.

The preparation of a transformed organism (or a transformed cell or tissue) requires that the corresponding DNA (e.g. the transformation vector) or RNA is introduced into the corresponding host cell. For this process which is referred to as transformation (or transduction or transfection), a multiplicity of methods and vectors are available (Keown et al. (1990) Methods in Enzymology 185:527-537; Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, pp. 71-119 (1993); White FF (1993) Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Editors: Kung and Wu R, Academic Press, 15-38; Jenes B et al. (1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, editors: Kung and R. Wu, Academic Press, pp.128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225; Halford NG, Shewry PR (2000) Br Med Bull 56(1):62-73).

For example, the DNA or RNA may be introduced directly by microinjection (WO 92/09696, WO 94/00583, EP-A 0 331 083, EP-A 0 175 966) or by bombardment with DNA or RNA-coded microparticles (biolistic processes using the gene gun "particle bombardment"; US 5,100,792; EP-A 0 444 882; EP-A 0 434 616; Fromm ME et al. (1990) Bio/Technology 8(9):833-9; Gordon-Kamm et al. (1990) Plant Cell 2:603). The cell may also be permeabilized chemically, for

example with polyethylene glycol, so as to enable the DNA to reach the cell by means of diffusion. The DNA may also take place by means of protoplast fusion to other DNA-containing units such as minicells, cells, lysosomes or liposomes (Freeman et al. (1984) Plant Cell Physiol. 29:1353ff; US 4,536,475). Electroporation is another suitable method for introducing DNA, in which the cells are permeabilized reversibly by an electric impulse (EP-A 290 395, WO 87/06614). Further processes comprise the calcium-phosphate-mediated transformation, DEAE-dextran-mediated transformation, the incubation of dry embryos in DNA-containing solution or other methods of direct introduction of DNA (DE 4 005 152, WO 90/12096, US 4,684,611). Appropriate processes have been described (e.g. in Bilang et al. (1991) Gene 100:247-250; Scheid et al. (1991) Mol Gen Genet 228:104-112; Guerche et al. (1987) Plant Science 52:111-116; Neuhause et al. (1987) Theor Appl Genet 75:30-36; Klein et al. (1987) Nature 327:70-73; Howell et al. (1980) Science 208:1265; Horsch et al.(1985) Science 227:1229-1231; DeBlock et al. (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)). Physical methods of introducing DNA into plant cells have been reviewed by Oard (1991) Biotech Adv 9:1-11.

In the case of these "direct" transformation methods, no particular requirements are made on the plasmid used. It is possible to use simple plasmids such as those of the pUC series, pBR322, M13mp series, pACYC184 etc.

Besides these "direct" transformation techniques, transformation may also be carried out by bacterial infection by means of Agrobacterium (e.g. EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611).

Transformation is preferably carried out by means of agrobacteria which contain disarmed Ti-plasmid vectors, using the latters' natural ability to transfer genes to plants (EP-A 0 270 355; EP-A 0 116 718). Agrobacterium transformation is widespread for transforming dicotyledones, but is also increasingly applied to monocotyledones (Toriyama et al. (1988) Bio/Technology 6: 1072-1074; Zhang et al. (1988) Plant Cell Rep 7:379-384; Zhang et al. (1988) Theor Appl Genet 76:835-840; Shimamoto et al. (1989) Nature 338:274-276; Datta et al. (1990) Bio/Technology 8: 736-740; Christou et al. (1991) Bio/Technology 9:957-962; Peng et al. (1991) International Rice Research Institute, Manila, Philippines

563-574; Cao et al. (1992) Plant Cell Rep 11:585-591; Li et al. (1993) Plant Cell Rep 12:250-255; Rathore et al. (1993) Plant Mol Biol 21:871-884; Fromm et al. (1990) Bio/Technology 8:833-839; Gordon-Kamm et al. (1990) Plant Cell 2:603-618; D'Halluin et al. (1992) Plant Cell 4:1495-1505; Walters et al. (1992) Plant Mol Biol 18:189-200; Koziel et al. (1993) Biotechnology 11:194-200; Vasil IK (1994) Plant Mol Biol 25:925-937; Weeks et al. (1993) Plant Physiol 102:1077-1084; Somers et al. (1992) Bio/Technology 10:1589-1594; WO 92/14828; Hiei et al. (1994) Plant J 6:271-282).

The strains most often used for agrobacterial transformation, Agrobacterium tumefaciens or Agrobacterium rhizogenes, contain a plasmid (Ti and Ri plasmids, respectively), which is transferred to the plant after agrobacterial infection. Part of this plasmid, called T-DNA (transferred DNA), is integrated into the genome of the plant cell. Alternatively, Agrobacterium may also transfer binary vectors (mini Ti plasmids) to plants and integrate them into the genome of said plants.

The application of Agrobacterium tumefaciens to the transformation of plants, using tissue culture explants, has been described (inter alia, Horsch RB et al. (1985) Science 225:1229ff; Fraley et al. (1983) Proc Natl Acad Sci USA 80: 4803-4807; Bevans et al. (1983) Nature 304:184-187). Many Agrobacterium tumefaciens strains are capable of transferring genetic material, such as, for example, the strains EHA101[pEHA101], EHA105[pEHA105], LBA4404[pAL4404], C58C1[pMP90] and C58C1[pGV2260] (Hood et al. (1993) Transgenic Res 2:208-218; Hoekema et al. (1983) Nature 303:179-181; Koncz and Schell (1986) Gen Genet 204:383-396; Deblaere et al. (1985) Nucl Acids Res 13: 4777-4788).

When using agrobacteria, the expression cassette must be integrated into special plasmids, either a shuttle or intermediate vector or a binary vector. When using a Ti or Ri plasmid for transformation, then at least the right border, but usually the right and left borders of the Ti or Ri plasmid T-DNA are connected as a flanking region to the expression cassette to be introduced. Preference is given to using binary vectors. Binary vectors may replicate both in E. coli and in agrobacteria and contain the components required for transfer into a plant system. They normally contain a selection marker gene for selection of transformed plants (e.g. the nptII gene which imparts a resistance to kanamycin) and a linker or polylinker flanked by the right and left T-DNA border sequences. They contain moreover, outside the T-DNA border sequence, also a selection marker which enables transformed E. coli and/or agrobacteria to be selected

(e.g. the nptIII gene which imparts a resistance to kanamycin). Corresponding vectors may be transformed directly into Agrobacterium (Holsters et al. (1978) Mol Gen Genet 163:181-187).

Binary vectors are based, for example, on "broad host range" plasmids such as pRK252 (Bevan et al. (1984) Nucl Acid Res 12,8711-8720) and pTJS75 (Watson et al. (1985) EMBO J 4(2):277-284). A large group of the binary vectors used is derived from pBIN19 (Bevan et al. (1984) Nucl Acid Res 12:8711-8720). Hajdukiewicz et al. developed a binary vector (pPZP) which is smaller and more efficient than the previously customary vectors (Hajdukiewicz et al. (1994) Plant Mol Biol 25:989-994). Improved and particularly preferred binary vector systems for Agrobacterium-mediated transformation are described in WO 02/00900.

The agrobacteria transformed with a vector of this kind may then be used in the known manner for transforming plants, in particular crop plants such as, for example, oilseed rape, for example by bathing wounded leaves or leaf sections in an agrobacterial solution and subsequently culturing them in suitable media. The transformation of plants by agrobacteria has been described (White FF, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38; Jenes B et al.(1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, pp.128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225). Transgenic plants may be regenerated in the known manner from the transformed cells of the wounded leaves or leaf sections.

Different explants, cell plants, tissues, organs, embryos, seeds, microspores or other unicellular or multicellular cellular structures derived from a plant organism may be used for transformation. Transformation processes adjusted to the particular explants, cultures or tissues are known to the skilled worker. Examples which may be mentioned are: shoot internodes (Fry J et al. (1987) Plant Cell Rep. 6:321-325), hypocotyls (Radke SE et al. (1988) Theor Appl Genet 75:685-694; Schröder M et al. (1994) Physiologia Plant 92: 37-46.; Stefanov I et al. (1994) Plant Sci. 95:175-186; Weier et al. (1997) Fett/Lipid 99:160-165), cotyledonous petioles (Meloney MM et al. (1989) Plant Cell Rep 8:238-242; Weier D et al. (1998) Molecular Breeding 4:39-46), microspores and proembryos (Pechnan (1989) Plant Cell Rep. 8:387-390) and flower stalks (Boulter ME et al. (1990) Plant Sci 70:91-99; Guerche P et al. (1987) Mol Gen Genet 206:382-386). In the case

of a direct gene transfer, mesophyll protoplasts (Chapel PJ & Glimelius K (1990) Plant Cell Rep 9: 105-108; Golz et al. (1990) Plant Mol Biol 15:475-483) or else hypocotyl protoplasts (Bergmann P & Glimelius K (1993) Physiologia Plant 88:604-611) and microspores (Chen JL et al. (1994) Theor Appl Genet 88:187-192; Jonesvilleneuve E et al. (1995) Plant Cell Tissue and Organ Cult 40:97-100) and shoot sections (Seki M et al. (1991) Plant Mol Biol 17:259-263) may be employed successfully.

Stably transformed cells, i.e. those which contain the introduced DNA integrated into the DNA of the host cell, may be selected from untransformed cells by using the selection process of the invention. The plants obtained may be grown and crossed in the usual way. Preferably, two or more generations should be cultured in order to ensure that the genomic integration is stable and can be inherited.

As soon as a transformed plant cell has been prepared, it is possible to obtain a complete plant by using proceses known to the skilled worker. This involves, for example, starting from callus cultures, individual cells (e.g. protoplasts) or leaf disks (Vasil et al. (1984) Cell Culture and Somatic Cel Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press; Weissbach and Weissbach (1989) Methods for Plant Molecular Biology, Academic Press). It is possible to induce from these still undifferentiated callus cell masses the formation of shoot and root in the known manner. The seedlings obtained may be planted out and grown. Appropriate processes have been described (Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al. (1995) Plant Cell Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533).

The efficacy of expressing the transgenically expressed nucleic acids may be determined, for example, *in vitro* by shoot-meristem propagation using any of the selection methods described above. Moreover, changes in the type and level of expression of a target gene and the effect on the phenotype of the plant may be tested in greenhouse experiments using test plants.

The process of the invention is preferably used within the framework of plant biotechnology for generating plants having advantageous properties. The "nucleic acid sequence to be inserted" into the genome of the plant cell or the plant organism preferably comprises at least one expression cassette, said expression cassette being able to express, under the control of a promoter functional in plant cells or plant organisms, an RNA and/or a

protein which do not cause reduction of the expression, amount, activity and/or function of a marker protein but, particularly preferably, impart to the plant genetically altered in this way an advantageous phenotype. Numerous genes and proteins which may be used for achieving an advantageous phenotype, for example for the increase in quality of foodstuff or for producing particular chemicals or pharmaceuticals (Dunwell JM (2000) J Exp Bot 51 Spec No:487-96) are known to the skilled worker.

Thus it is possible to improve the suitability of the plants or the seeds thereof as foodstuff or feedstuff, for exmaple by altering the compositions and/or the content of metabolites, in particular proteins, oils, vitamins and/or starch. It is also possible to increase the growth rate, yield or resistance to biotic or abiotic stress factors. Advantageous effects may be achieved both by transgenic expression of nucleic acids or proteins and by targeted reduction of the expression of endogenous genes, with respect to the phenotype of the transgenic plant. The advantageous effects which may be achieved in the transgenic plant comprise, for example:

- increased resistance to pathogens (biotic stress)
- increased resistance to environmental factors such as heat, cold, frost, drought, UV light, oxidative stress, wetness, salt, etc. (abiotic stress)
- increased yield
- improved quality, for example increased nutritional value, increased storability

The invention further relates to the use of the transgenic plants prepared according to the process of the invention and of the cells, cell cultures, plants or propagation material such as seeds or fruits derived from said plants, for preparing foodstuff or feedstuff, pharmaceuticals or fine chemicals such as, for example, enzymes, vitamins, amino acids, sugars, fatty acids, natural and synthetic flavorings, aroma substances and colorants. Particular preference is given to the production of triacyl glycerides, lipids, oils, fatty acids, starch, tocopherols and tocotrienols and also carotenoids. Genetically modified plants of the invention, which may be consumed by humans and animals may also be used as foodstuff or feedstuff, for example, directly or after preparation known per se.

As already mentioned above, the process of the invention comprises in a particularly advantageous embodiment, in a process step downstream of the selection, the deletion of the sequence coding for the marker protein (e.g. mediated by recombinase or as described in WO03/004659) or the elimination by crossing and/or segregation of said sequences. (It is obvious to the skilled worker that, for this purpose, the nucleic acid sequence integrated into the genome and the sequence coding for the marker protein should have a separate chromosomal locus in the transformed cells. This, however, is the case in the majority of the resulting plants, merely for reasons of statistics). This procedure is particularly advantageous if the marker protein is a transgene which otherwise does not occur in the plant to be transformed. Although the resulting plant may still possibly contain the compound for reducing the expression, amount, activity and/or function of the marker protein, said compound would have no longer any "counterpart" in the form of said marker protein, and thus would have no effect. This is particularly the case if the marker protein is derived from a non-plant organism and/or is synthetic (for example the codA protein). It is, however, also possible to use plant marker proteins from other plant species, which otherwise do not occur in the cell to be transformed (i.e. if not introduced as transgene). Said marker proteins are referred to as "nonendogenous" marker proteins within the scope of the present invention.

Very particularly advantageously, the compound for reducing the expression, amount, activity and/or function of the marker protein is an RNA. After deletion or elimination by crossing/segregation, the resulting transgenic plant would have no longer any unnecessary (and, if appropriate, undesired) foreign protein. The sole foreign protein would be possibly the protein resulting from the nucleic acid sequence inserted into the genome. For reasons of product approval, this embodiment is particularly advantageous. As described above, said RNA may be an antisense RNA or, particularly preferably, a double-stranded RNA. It may be expressed separately from the RNA coding for the target protein but also, possibly, on the same strand as the latter.

In summary, the particularly advantageous embodiment comprises the following features:

A process for preparing transformed plant cells or organisms, which comprises the following steps:

a) transforming a population of plant cells which comprises at least one non-endogenous (preferably non-plant) marker pro-

tein capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population, with at least one nucleic acid sequence to be inserted in combination with at least one nucleic acid sequence coding for a ribonucleic acid sequence capable of reducing the expression, amount, activity and/or function of said marker protein, and

b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and

c) selecting transformed plant cells (and/or populations of plant cells, such as plant tissues or plants) whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells, and

d) regenerating fertile plants, and

e) eliminating by crossing the nucleic acid sequence coding for the marker protein and isolating fertile plants whose genome contains said nucleic acid sequence but does not contain any longer the sequence coding for the marker protein.

Sequences

SEQ ID NO: 1 Nucleic acid sequence coding for E. coli cytosine deaminase (codA)

SEQ ID NO: 2 amino acid sequence coding for E. coli cytosine deaminase (codA)

SEQ ID NO: 3 Nucleic acid sequence coding for E. coli cytosine deaminase (codA), with modified start codon (GTG/ATG) for expression in eukaryotes

SEQ ID NO: 4 Amino acid sequence coding for E. coli cytosine deaminase (codA), with modified start codon (GTG/ATG) for expression in eukaryotes

SEQ ID NO: 5 Nucleic acid sequence coding for Streptomyces griseolus cytochrome P450-SU1 (suaC)

SEQ ID NO: 6 Amino acid sequence coding for Streptomyces griseolus cytochrome P450-SU1 (suaC)

SEQ ID NO: 7 Nucleic acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (tms2)

SEQ ID NO: 8 Amino acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (tms2)

SEQ ID NO: 9 Nucleic acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (tms2)

SEQ ID NO: 10 Amino acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (tms2)

SEQ ID NO: 11 Nucleic acid sequence coding for Xanthobacter autotrophicus haloalkane dehalogenase (dhlA)

SEQ ID NO: 12 Amino acid sequence coding for Xanthobacter autotrophicus haloalkane dehalogenase (dhlA)

SEQ ID NO: 13 Nucleic acid sequence coding for Herpes simplex Virus 1 thymidine kinase

SEQ ID NO: 14 Amino acid sequence coding for Herpes simplex Virus 1 thymidine kinase

SEQ ID NO: 15 Nucleic acid sequence coding for Herpes simplex Virus 1 thymidine kinase

SEQ ID NO: 16 Amino acid sequence coding for Herpes simplex Virus 1 thymidine kinase

SEQ ID NO: 17 Nucleic acid sequence coding for Toxoplasma gondii hypoxanthine-xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 18 Amino acid sequence coding for Toxoplasma gondii hypoxanthine-xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 19 Nucleic acid sequence coding for E. coli xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 20 Amino acid sequence coding for E. coli xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 21 Nucleic acid sequence coding for E. coli xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 22 Amino acid sequence coding for E. coli xanthine-guanine phosphoribosyl transferase

SEQ ID NO: 23 Nucleic acid sequence coding for E. coli purine nucleoside phosphorylase (deoD)

SEQ ID NO: 24 Nucleic acid sequence coding for E. coli purine nucleoside phosphorylase (deoD)

SEQ ID NO: 25 Nucleic acid sequence coding for Burkholderia caryophylli phosphonate monoester hydrolase (pehA)

SEQ ID NO: 26 Amino acid sequence coding for Burkholderia caryophylli phosphonate monoester hydrolase (pehA)

SEQ ID NO: 27 Nucleic acid sequence coding for Agrobacterium rhizogenes tryptophan oxygenase (aux1)

SEQ ID NO: 28 Amino acid sequence coding for Agrobacterium rhizogenes tryptophan oxygenase (aux1)

SEQ ID NO: 29 Nucleic acid seuence coding for Agrobacterium rhizogenes indoleacetamide hydrolase (aux2)

SEQ ID NO: 30 Amino acid seuence coding for Agrobacterium rhizogenes indoleacetamide hydrolase (aux2)

SEQ ID NO: 31 Nucleic acid sequence coding for Agrobacterium tumefaciens tryptophan oxygenase (aux1)

SEQ ID NO: 32 Amino acid sequence coding for Agrobacterium tumefaciens tryptophan oxygenase (aux1)

SEQ ID NO: 33 Nucleic acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (aux2)

SEQ ID NO: 34 Amino acid sequence coding for Agrobacterium tumefaciens indoleacetamide hydrolase (aux2)

SEQ ID NO: 35 Nucleic acid sequence coding for Agrobacterium vitis indoleacetamide hydrolase (aux2)

SEQ ID NO: 36 Amino acid sequence coding for Agrobacterium vitis indoleacetamide hydrolase (aux2)

SEQ ID NO: 37 Nucleic acid sequence coding for Arabidopsis thaliana 5-methylthioribose kinase (mtrK)

SEQ ID NO: 38 Amino acid sequence coding for Arabidopsis thaliana 5-methylthioribose kinase (mtrK)

SEQ ID NO: 39 Nucleic acid sequence coding for Klebsiella pneumoniae 5-methylthioribose kinase (mtrK)

SEQ ID NO: 40 Amino acid sequence coding for Klebsiella pneumoniae 5-methylthioribose kinase (mtrK)

SEQ ID NO: 41 Nucleic acid sequence coding for Arabidopsis thaliana alcohol dehydrogenase (adh)

SEQ ID NO: 42 Amino acid sequence coding for Arabidopsis thaliana alcohol dehydrogenase (adh)

SEQ ID NO: 43 Nucleic acid sequence coding for Hordeum vulgare (barley) alcohol dehydrogenase (adh)

SEQ ID NO: 44 Amino acid sequence coding for Hordeum vulgare (barley) alcohol dehydrogenase (adh)

SEQ ID NO: 45 Nucleic acid sequence coding for Oryza sativa (rice) alcohol dehydrogenase (adh)

SEQ ID NO: 46 Amino acid sequence coding for Oryza sativa (rice) alcohol dehydrogenase (adh)

SEQ ID NO: 47 Nucleic acid sequence coding for Zea mays (corn) alcohol dehydrogenase (adh)

SEQ ID NO: 48 Amino acid sequence coding for Zea mays (corn) alcohol dehydrogenase (adh)

SEQ ID NO: 49 Nukleic acid sequence coding for a sense RNA fragment of E. coli cytosine deaminase (codARNAi-sense)

SEQ ID NO: 50 Oligonucleotide primer codA5'HindIII
5'-AAGCTTGGCTAACAGTGTCGAATAACG-3'

SEQ ID NO: 51 Oligonucleotide primer codA3'SalI
5'-GTCGACGACAAAATCCCTTCCTGAGG-3'

SEQ ID NO: 52 Nucleic acid sequence coding for an antisense RNA fragment of E. coli cytosine deaminase (codARNAi-anti)

SEQ ID NO: 53 Oligonucleotide primer codA5'EcoRI
5'-GAATTCGGCTAACAGTGTCGAATAACG-3'

SEQ ID NO: 54 Oligonucleotide primer codA3'BamHI
5'-GGATCCGACAAAATCCCTTCCTGAGG-3'

SEQ ID NO: 55 Vector construct pBluKS-nitP-STLS1-35S-T

SEQ ID NO: 56 Expression vector pSUN-1

SEQ ID NO: 57 Transgenic expression vector pSUN-1-codA-RNAi

SEQ ID NO: 58 Transgenic expression vector pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT

SEQ ID NO: 59 Nukleic acid sequence coding for 5-methylthioribose kinase (mtrK) from corn (Zea mays); fragment

SEQ ID NO: 60 Amino acid sequence coding for 5-methylthioribose kinase (mtrK) from corn (Zea mays); fragment

SEQ ID NO: 61 Nucleic acid sequence coding for 5-methylthioribose kinase (mtrK) from oilseed rape (Brassica napus), fragment

SEQ ID NO: 62 Amino acid sequence coding for 5-methylthioribose kinase (mtrK) from oilseed rape (Brassica napus), fragment

SEQ ID NO: 63 Nucleic acid sequence coding for 5-methylthioribose kinase (mtrK) from oilseed rape (Brassica na-

pus), fragment

SEQ ID NO: 64 Amino acid sequence coding for 5-methylthioribose kinase (mtrK) from oilseed rape (Brassica napus), fragment

SEQ ID NO: 65 Nucleic acid sequence coding for 5-methylthioribose kinase (mtrK) from rice (Oryza sativa), fragment

SEQ ID NO: 66 Amino acid sequence coding for 5-methylthioribose kinase (mtrK) from rice (Oryza sativa), fragment

SEQ ID NO: 67 Nucleic acid sequence coding for 5-methylthioribose kinase (mtrK) from soybean (Glycine max), fragment

SEQ ID NO: 68 Amino acid sequence coding for 5-methylthioribose kinase (mtrK) from soybean (Glycine max), fragment

SEQ ID NO: 69 Oligonucleotide primer codA5'C-term
5'-CGTGAATACGGCGTGGAGTCG-3'

SEQ ID NO: 70 Oligonucleotide primer codA3'C-term
5'-CGGCAGGATAATCAGGTTGG-3'

SEQ ID NO: 71 Oligonucleotide primer 35sT 5' primer
5'-GTCAACGTAACCAACCCTGC-3'

Figures

Fig.1: Inactivation of the marker protein gene by means of introducing a recombinase

P:	promoter
MP:	Sequence coding for a marker protein
R1/R2:	Recombinase recognition sequences
R:	Recombinase or sequence coding for recombinase.

In a preferred embodiment, the marker protein gene is inactivated by introducing a sequence-specific recombinase. Preference is given to its expressing the recombinase, as depicted here, starting from an expression cassette.

The marker protein gene is flanked by recognition sequences for sequence-specific recombinases, with sequences of said marker protein gene being deleted by introducing said recombinase and thus said marker protein gene being inactivated.

Fig.2-A: Inactivation of the marker protein gene by the action of a sequence-specific nuclease

P:	promoter
DS:	Recognition sequence for targeted induction of DNA double-strand breaks
MP-DS-MP':	Sequence coding for a marker protein, comprising a DS
nDS:	Inactivated DS
E:	Sequence-specific enzyme for targeted induction of DNA double-strand breaks

The marker protein gene may be established by a targeted mutation or deletion in the marker protein gene, for example by sequence-specific induction of DNA double-strand breaks at a recognition sequence for targeted induction of DNA double-strand breaks in or close to the marker protein gene (P-MP). The double-strand break may occur in the coding region or else the noncoding (such as, for example, the promoter) region, induces an illegitimate recombination (nonhomologous DNA-end joining) and thus, for example, a shift in the reading frame of said marker protein.

Fig.2-B: Inactivation of the marker protein gene by the action of a sequence-specific nuclease

P:	promoter
DS:	Recognition sequence for targeted induction of DNA double-strand breaks
MP:	Sequence coding for a marker protein
nDS:	Inactivated DS
E:	Sequence-specific enzyme for targeted induction of DNA double-strand breaks

The marker protein gene may be established by a targeted deletion by sequence-specific induction of more than one sequence-specific DNA double-strand break in or close to said marker protein gene. The double-strand breaks may occur in the coding region or else the noncoding (such as, for example, the promoter) region and induce a deletion in the marker protein gene. The marker protein gene is preferably flanked by DS sequences and is completely deleted by the action of enzyme E.

Fig. 3: Inactivation of the marker protein gene by inducing an intramolecular homologous recombination, due to the action of a sequence-specific nuclease

A/A':	Sequences with a sufficient length and homology to one another, in order to recombine with one another as a consequence of the induced double-strand break
P:	promoter
DS:	Recognition sequence for targeted induction of DNA double-strand breaks
MP:	Sequence coding for a marker protein
E:	Sequence-specific enzyme for targeted induction of DNA double-strand breaks

The marker protein gene may be inactivated by a deletion by means of intramolecular homologous recombination. Said homologous recombination may be initiated by sequence-specific induction of DNA double-strand breaks at a recognition sequence for targeted induction of DNA double-strand breaks in or close to the marker protein gene. The homologous recombination occurs between the sequences A and A' which have a sufficient length and homology to one another in order to recombine with one another as a consequence of the induced double-strand break. The recom-

bination causes a deletion of essential sequences of the marker protein gene.

Fig. 4: Inactivation of the marker protein gene by intermolecular homologous recombination

A/A':	Sequences with a sufficient length and homology to one another in order to recombine with one another
B/B':	Sequences with a sufficient length and homology to one another in order to recombine with one another
P:	promoter
I:	nucleic acid sequence/gene of interest to be inserted
MP:	Sequence coding for a marker protein

The marker protein gene (P-MP) may also be inactivated by a targeted insertion into the marker protein gene, for example by means of intermolecular homologous recombination. In this context, the region to be inserted is flanked on its 5' and 3' ends by nucleic acid sequences (A' and B', respectively), which have a sufficient length and homology to corresponding flanking sequences of the marker protein (A and B, respectively) in order to make possible a homologous recombination between A and A' and B and B'. The recombination causes a deletion of essential sequences of the marker protein gene.

Fig. 5: Inactivation of the marker protein gene by intermolecular homologous recombination due to the action of a sequence-specific nuclease

A/A':	Sequences with a sufficient length and homology to one another in order to recombine with one another
B/B':	Sequences with a sufficient length and homology to one another in order to recombine with one another
P:	promoter
I:	nucleic acid sequence/gene of interest to be inserted
MP:	Sequence coding for a marker protein
DS:	Recognition sequence for targeted induction of DNA double-strand breaks
E:	Sequence-specific enzyme for targeted

induction of DNA double-strand breaks

The marker protein gene may also be inactivated by a targeted insertion into the marker protein gene, for example by means of intermolecular homologous recombination. The homologous recombination may be initiated by sequence-specific induction of DNA double-strand breaks at a recognition sequence for targeted induction of DNA double-strand breaks in or close to the marker protein gene. In this context, the region to be inserted is flanked at its 5' and 3' ends by nucleic acid sequences (A' and B', respectively) which have a sufficient length and homology to corresponding flanking sequences of the marker protein gene (A and B, respectively) in order to make possible a homologous recombination between A and A' and B and B'. The recombination causes a deletion of essential sequences of the marker protein gene.

Fig. 6: Vector map for pBluKS-nitP-STLS1-35S-T (SEQ ID NO: 55)

NitP: promoter of the A. thaliana nitrilaseI gene (GenBank Acc. No.: Y07648.2, Hillebrand et al. (1996) Gene 170:197-200)

STLS-1 intron: intron of the potato ST-LS1 gene (Vancanneyt GF et al. (1990) Mol Gen Genet 220(2):245-250).

35S-Term: Terminator of the 35S CaMV gene (cauliflower mosaic virus; Franck et al. (1980) Cell 21:285-294).

Cleavage sites of relevant restriction endonucleases are indicated with their particular cleavage position.

Fig. 7: Vector map for the transgenic expression vector pSUN-1-codA-RNAi (SEQ ID NO: 57)

NitP: promoter of the A. thaliana nitrilaseI gene (GenBank Acc. No.: Y07648.2, Hillebrand et al. (1996) Gene 170:197-200)

STLS-1 intron: intron of the potato ST-LS1 gene (Vancanneyt GF et al. (1990) Mol Gen Genet 220(2):245-250).

35S-Term: Terminator of the 35S CaMV gene (cauliflower mosaic virus; Franck et al. (1980) Cell 21:285-294).

codA-sense: Nucleic acid sequence coding for a sense RNA fragment of E. coli cytosine deaminase (codARNAi-sense; SEQ ID NO: 49)

codA-anti: Nucleic acid sequence coding for an antisense RNA fragment of E. coli cytosine deaminase (codARNAi-anti; SEQ ID NO: 52)

LB/RB: Left and, respectively, right boundaries of Agrobacterium T-DNA

Cleavage sites of relevant restriction endonucleases are indicated with their particular cleavage position. Further elements represent customary elements of a binary Agrobacterium vector (aadA; ColE1; repA)

Fig. 8: Vector map for the transgenic expression vector pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT (SEQ ID NO: 58)

NitP: promoter of the A. thaliana nitrilaseI gene (GenBank Acc. No.: Y07648.2, Hillebrand et al. (1996) Gene 170:197-200)

STLS-1 intron: intron of the potato ST-LS1 gene (Vancanneyt GF et al. (1990) Mol Gen Genet 220(2):245-250).

35S-Term: Terminator of the 35S CaMV gene (cauliflower mosaic virus; Franck et al. (1980) Cell 21:285-294).

codA-sense: Nucleic acid sequence coding for a sense RNA fragment of E. coli cytosine deaminase (codARNAi-sense; SEQ ID NO: 49)

codA-anti: Nucleic acid sequence coding for an antisense RNA fragment of E. coli cytosine deaminase (codARNAi-anti; SEQ ID NO: 52)

Left border/right border: Left and, respectively, right boundaries of Agrobacterium T-DNA

Cleavage sites of relevant restriction endonucleases are indicated with their particular cleavage position. Further elements represent customary elements of a binary Agrobacterium vector (aadA; ColE1; repA)

Fig.9a-b: Sequence comparison of various 5-methylthioribose (MTR) kinases from various organisms, in particular plant organisms. Sequences from Klebsiella pneumoniae, Clostridium tetani, Arabidopsis thaliana (A.thaliana), oilseed rape (Brassica napus), soybean (Soy-1), rice (Oryza sativa-1) and also the consensus sequence (Consensus) are shown. Homologous regions can be readily deduced from the consensus sequence.

Exemplary embodiments

General methods

The chemical synthesis of oligonucleotides may be carried out, for example, in the known manner by using the phosphoamide method (Voet, Voet, 2nd Edition, Wiley Press New York, pages 896-897). The cloning steps carried out within the scope of the present invention, such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking of DNA fragments, transformation of E. coli cells, cultivation of bacteria, propagation of phages and sequence analysis of recombinant DNA, are carried out as described in Sambrook et al. (1989) Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6. The sequencing of recombinant DNA molecules was carried out using a laser fluorescence DNA sequencer from ABI, according to the method of Sanger (Sanger et al. (1977) Proc Natl Acad Sci USA 74:5463-5467).

Example 1: Preparation of codA fragments

First, a truncated nucleic acid variant of the *codA* gene, modified by the addition of recognition sequences of the restriction enzymes HindIII and SalI, is prepared using the PCR technique. For this purpose, part of the *codA* gene (GeneBank Acc. No.: S56903; SEQ ID NO: 1) is amplified from the E. coli source organism by means of the polymerase chain reaction (PCR) using a sense-specific primer (codA5'HindIII; SEQ ID NO: 50) and an antisense-specific primer (codA3'SalI; SEQ ID NO: 51).

codA5'HindIII: 5'-AAGCTTGGCTAACAGTGTCGAATAACG-3' (SEQ ID NO: 50)

codA3'SalI: 5'-GTCGACGACAAAATCCCTTCCTGAGG-3' (SEQ ID NO: 51)

The PCR was carried out in 50 µl reaction mixture which contained:

- 2 µl (200 ng) of *E. coli* genomic DNA
- 0.2 mM dATP, dTTP, dGTP, dCTP
- 1.5 mM $Mg(OAc)_2$
- 5 µg of bovine serum albumin
- 40 pmol of "codA5'HindIII" primer
- 40 pmol of "codA3'SalI" primer
- 15 µl of 3.3× rTth DNA Polymerase XLPuffer (PE Applied Biosystems)

- 5U of rTth DNA Polymerase XL (PE Applied Biosystems)

The PCR is carried out under the following cycle conditions:

Step 1: 5 minutes 94°C (denaturation)
Step 2: 3 seconds 94°C
Step 3: 1 minute 60°C (annealing)
Step 4: 2 minutes 72°C (elongation)

30 repeats of steps 2 to 4

Step 5: 10 minutes 72°C (post elongation)
Step 6: 4°C (waiting loop)

The amplicon (codARNAi-sense; SEQ ID NO: 49) is cloned using standard methods into the PCR cloning vector pGEM-T (Promega). The identity of the amplicon generated is confirmed by sequencing using the M13F (-40) primer.

Another truncated fragment of the *codA* gene, modified by the addition of recognition sequences of the restriction enzymes Eco-RI and BamHI, is amplified using a sense-specific primer (codA5'EcoRI; SEQ ID NO: 53) and an antisense-specific primer (codA3'BamHI; SEQ ID NO: 54).

codA5'EcoRI: 5'-GAATTCGGCTAACAGTGTCGAATAACG-3' (SEQ ID NO: 53)

codA3'BamHI: 5'-GGATCCGACAAAATCCCTTCCTGAGG-3' (SEQ ID NO: 54)

The PCR was carried out in 50 µl reaction mixture which contained:

- 2 µl (200 ng) of *E. coli* genomic DNA
- 0.2 mM dATP, dTTP, dGTP, dCTP
- 1.5 mM $Mg(OAc)_2$
- 5 µg of bovine serum albumin
- 40 pmol of "codA5'EcoRI" primer
- 40 pmol of "codA3'BamHI" primer
- 15 µl of 3.3x rTth DNA Polymerase XLPuffer (PE Applied Biosystems)
- 5U of rTth DNA Polymerase XL (PE Applied Biosystems)

The PCR is carried out under the following cycle conditions:

Step 1: 5 minutes 94°C (denaturation)
Step 2: 3 seconds 94°C
Step 3: 1 minute 60°C (annealing)
Step 4: 2 minutes 72°C (elongation)

30 repeats of steps 2 to 4

Step 5: 10 minutes 72°C (post elongation)
Step 6: 4°C (waiting loop)

The amplicon (codARNAi-anti; SEQ ID NO: 52) is cloned using standard methods into the PCR cloning vector pGEM-T (Promega). The identity of the amplicon generated is confirmed by sequencing using the M13F (-40) primer.

Example 2 Preparation of the transgenic expression vector for expressing a codA double-stranded RNA

The codA fragments generated in example 1 are used for preparing a DNA construct suitable for expressing a double-stranded codA RNA (pSUN-codA-RNAi). The construct is suitable for reducing the steady-state RNA level of the *codA* gene in transgenic plants and, as a result therefrom, suppressing *codA* gene expression by using the double-strand RNA interference (dsRNAi) technique. For this purpose, the *codA* RNAi cassette is first constructed in the plasmid pBluKS-nitP-STLS1-35S-T and then, in a further cloning step, completely transferred to the pSUN-1 plasmid.

The vector pBluKS-nitP-STLS1-35S-T (SEQ ID NO: 55) is a derivative of pBluescript KS (Stratagene) and contains the promoter of the A. thaliana nitrilaseI gene (GenBank Acc. No.: Y07648.2, nucleotides 2456 to 4340, Hillebrand et al. (1996) Gene 170:197-200), the STLS-1 intron (Vancanneyt GF et al. (1990) Mol Gen Genet 220(2):245-250), restriction cleavage sites flanking the intron on its 5' and 3' sides and enabling DNA fragments to be inserted in a directed manner, and the terminator of the 35S CaMV gene (cauliflower mosaic virus; Franck et al. (1980) Cell 21:285-294). Using these restriction cleavage sites (HindIII, SalI, EcoRI, BamHI), the fragments codARNAi-sense (SEQ ID NO: 49) and codARNAi-anti (SEQ ID NO: 52) are inserted into said vector, thereby producing the finished codA RNAi cassette.

For this purpose, the codA sense fragment (codARNAi-sense SEQ ID NO: 49) is first excised from the pGEM-T vector, using the enzymes HindIII and SalI, isolated and ligated into the pBluKS-

nitP-STLS1-35S-T vector under standard conditions. This vector had previously been cleaved using the restriction enzymes HindIII and SalI. Correspondingly positive clones are identified by analytical restriction digest and sequencing.

The vector obtained (pBluKS-nitP-codAsense-STLS1-35S-T) is digested using the restriction enzymese BamHI and EcoRI. The codA-anti fragment (codARNAi-anti; SEQ ID NO: 52) is excised from the corresponding pGEM-T vector, using BamHI and EcoRI, isolated and ligated into the cut vector under standard conditions. Correspondingly positive clones which contain the complete codA-RNAi cassette (pBluKS-nitP-codAsense-STLS1-codAanti-35S-T) are identified by analytical restriction digest and sequencing.

The codA-RNAi cassette is transferred into the pSUN-1 vector (SEQ ID NO: 56) by using the SacI and KpnI restriction cleavage sites flanking the cassette. The resulting vector pSUN1-codA-RNAi (see Fig. 7; SEQ ID NO: 57) is used for transforming transgenic *A.thaliana* plants which express an active *codA* gene (see below). The plant expression vector pSUN-1 is particularly suitable within the scope of the process of the invention, since it does not contain any other positive selection marker.

The resulting vector, pSUN1-codA-RNAi, enables an artificial *codA*-dsRNA variant consisting of two identical nucleic acid elements which are separated by an intron and inverted to one another to be constitutively expressed. Transcription of this artificial *codA*-dsRNA variant results in the formation of a double-stranded RNA molecule, owing to the complementarity of the inverted nucleic acid elements. The presence of this molecule induces the suppression of *codA* gene expression (accummulation of RNA) by means of double-strand RNA interference.

Example 4: Preparation of transgenic *Arabidopis thaliana* plants

Transgenic Arabidopsis thaliana plants which express transgenically the E. coli codA gene as a marker protein ("A. thaliana-[codA]"), were prepared as described (Kirik et al. (2000) EMBO J 19(20):5562-6).

The A. thaliana-[codA] plants are transformed with an Agrobacterium tumefaciens strain (GV3101 [pMP90]) on the basis of a modified vacuum infiltration method (Clough S & Bent A (1998) Plant J 16(6):735-43; Bechtold N et al. (1993) CR Acad Sci Paris 1144(2):204-212). The *Agrobacterium tumefaciens* cells used have previously been transformed with the DNA construct described

(pSUN1-codA-RNAi). In this way, double transgenic A. thaliana-[codA] plants are generated which express an artificial codA double-stranded RNA under the control of the constitutive nitrilase1 promoter. Expression of the codA gene is suppressed as a consequence of the dsRNAi effect induced by the presence of this artificial codA-dsRNA. Said double transgenic plants may be identified owing to their regained ability to grow in the presence of 5-fluorocytosine in the culture medium.

Seeds of primary transformants are selected on the basis of the regained ability to grow in the presence of 5-fluorocytosine. For this purpose, the T1 seeds of the primary transformants are laid out on selection medium containing 200 µg/ml 5-fluorocytosine. These selection plates are incubated under long-day conditions (16 h of light, 21°C/8 h of darkness, 18°C). Seedlings which develop normally in the presence of 5-fluorocytosine are separated after 7 days and transferred to new selection plates. These plates are incubated for another 14 under unchanged conditions. The resistant seedlings are then transplanted into soil and cultured under short-day conditions (8 h of light, 21°C/16 h of darkness, 18°C). After 14 days, the young plants are transferred to the greenhouse and cultured under short-day conditions.

Example 5: Preparation of a plant transformation vector containing an expression cassette for expressing a double-stranded codA RNA and a plant selection marker

A plant selection marker consisting of a mutated variant of the A. thaliana Als gene, coding for the acetolactate synthase under the control of the promoter of the A. thaliana actin-2 gene (Meagher RB & Williamson RE (1994) The plant cytoskeleton. In The Plant Cytoskeleton (Meyerowitz, E. & Somerville, C., eds), pp. 1049-1084. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), and the octopine synthase terminator (GIELEN J et al.(1984) EMBO J 3:835-846) is inserted into pSUN1-codA-RNAi (see Fig. 7; SEQ ID NO: 57) (At.Act.-2-At.Als-R-ocsT).

For this purpose, the pSUN1-codA-RNAi vector is first linearized using the restriction enzyme Pvu II. Subsequently, a linear DNA fragment with blunt ends, coding for a mutated variant of the acetolactate synthase (Als-R gene), is ligated into said linearized vector under standard conditions. Prior to ligation, this DNA fragment has been digested with the restriction enzyme KpnI and the protruding ends have been converted into blunt ends by treatment with Pwo DNA polymerase (Roche) according to the

manufacturer's instructions. This mutated variant of the A. thaliana Als gene cannot be inhibited by herbicides of the imidazolinone type. By expressing this mutated A.tAls-R gene, the plants obtain the ability to grow in the presence of the herbicide Pursuit™. Correspondingly positive clones (pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT; SEQ ID NO: 57) are identified by analytical restriction digest and sequencing.

The vector obtained enables an artificial codA RNA variant (consisting of two identical nucleic acid elements which are separated by an intron and inverted to one another) and a mutated variant of the A. thaliana Als gene to be expressed constitutively. Transcription of this artificial codA RNA variant results in the formation of a double-stranded RNA molecule, owing to the complementarity of the inverted nucleic acid elements. The presence of this molecule induces the suppression of codA gene expression (accummulation of RNA) by means of double-strand RNA interference. Expression of the Als-R gene imparts to the plants the ability to grow in the presence of herbicides of the imidazolinone type.

Example 6: Preparation of transgenic Arabidopis thaliana plants

Transgenic Arabidopsis thaliana plants expressing the E. coli codA gene as a marker protein ("A.thaliana-[codA]") were prepared as described (Kirik et al.(2000) EMBO J 19(20):5562-6).

The A.thaliana-[codA] plants are transformed with an Agrobacterium tumefaciens strain (GV3101 [pMP90]) on the basis of a modified vacuum infiltration method (Clough S & Bent A (1998) Plant J 16(6):735-43; Bechtold N et al. (1993) CR Acad Sci Paris 1144(2):204-212). The Agrobacterium tumefaciens cells used have previously been transformed with the DNA construct described (pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT; SEQ ID NO: 57). In this way, double transgenic A. thaliana-[codA] plants are generated which additionally express an artificial codA double-stranded RNA and a herbicide-insensitive variant of the Als gene (Als-R) under the control of the constitutive nitrilasel promoter (A.thaliana-[codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT]). Expression of the codA gene is suppressed as a consequence of the dsRNAi effect induced by the presence of this artificial codA-dsRNA. These double transgenic plants may be identified owing to their regained ability to grow in the presence of 5-fluorocytosine in the culture medium. In addition, positively transformed plants can be selected owing to their ability to grow in the presence of the her-

bicide Pursuit in the culture medium.

For the purpose of selection, the T1 seeds of primary transformants are therefore laid out on selection medium containing 100 µg/ml 5-fluorocytosine. These selection plates are incubated under long-day conditions (16 h of light, 21°C/8 h of darkness, 18°C). Seedlings which develop normally in the presence of 5-fluorocytosine are separated after 28 days and transferred to new selection plates. These plates are incubated for another 14 days under unchanged conditions. The resistant seedlings are then transplanted into soil and cultured under short-day conditions (8 h of light, 21°C/16 h of darkness, 18°C). After a further 14 days, the young plants are transferred to the greenhouse and cultured under short-day conditions.

In addition, seeds of the primary transformants, owing to their ability to grow in the presence of the herbicide Pursuit™, may be selected. It is furthermore possible to carry out dual selection using the herbicide Pursuit™ and 5-fluorocytosine. For this purpose, the T1 seeds of primary transformants are laid out on selection medium containing the herbicide Pursuit™ at a concentration of 100 nM (in the case of dual selection, 100 µg/ml 5-fluorocytosine is likewise present). These selection plates are incubated under long-day conditions (16 h of light, 21°C/8 h of darkness, 18°C).

Seedlings which develop normally in the presence of Pursuit™ (Pursuit™ and 5-fluorocytosine) are separated after 28 days and transferred to new selection plates. These plates are incubated under unchanged conditions for another 14 days. The resistant seedlings are then transplanted into soil and cultured under short-day conditions (8 h of light, 21°C/16 h of darkness, 18°C). After 14 days, the young plants are transferred to the greenhouse and cultured under short-day conditions.

Example 7: Analysis of the double transgenic A. thaliana plants selected using 5-fluorocytosine and/or Pursuit (A.thaliana-[codA]-[codA-RNAi- At.Act.-2-At.Als-R-ocsT])

Integration of the T-DNA region of the vector used for transformation, pSUN1-codA-RNAi-A.tAls-R, into the genomic DNA of the starting plant (A.thaliana-[codA]) and the loss of codA-specific mRNA in these transgenic plants (A.thaliana-[codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT]) can be detected by applying Southern

analyses and PCR techniques or Northern analyses.

In order to carry out said analyses, total RNA and DNA are isolated from leaf tissue of the transgenic plants and suitable controls (using the RNeasy Maxi Kit (RNA) and Dneasy Plant Maxi Kit (genomic DNA), respectively, according to the manufacturer's information by Qiagen).

In the PCR analyses, the genomic DNA may be used directly as a basis (template) for the PCR. Total RNA is transcribed to cDNA prior to the PCR. The cDNA synthesis is carried out using the reverse transcriptase Superscript II (Invitrogen) according to the manufacturer's information.

Example 8: Detection of the reduction in the steady-state amount of codA RNA in the positively selected double transgenic plants (A.thaliana [codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT]) in comparison with the starting plants (A.thaliana [codA]) used for transformation, by means of cDNA synthesis with subsequent PCR amplification.

PCR amplification of the codA-specific cDNA:
The cDNA of the codA gene (ACCESSION S56903) may be amplified using a sense-specific primer (codA5'C-term SEQ ID NO: 69) and an antisense-specific primer (codA3'C-term SEQ ID NO: 70). The PCR conditions to be chosen are as follows:

The PCR was carried out in 50 µl reaction mixture which contained:

- 2 µl (200 ng) of cDNA from A.thaliana -[codA] or A.thaliana [codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT] plants
- 0.2 mM dATP, dTTP, dGTP, dCTP
- 1.5 mM $Mg(OAc)_2$
- 5 µg of bovine serum albumin
- 40 pmol of codA5'C-term SEQ ID NO: 69
- 40 pmol of codA3'C-term SEQ ID NO: 70
- 15 µl of 3.3x rTth DNA Polymerase XLPuffer (PE Applied Biosystems)
- 5U of rTth DNA Polymerase XL (PE Applied Biosystems)

The PCR was carried out under the following cycle conditions:
Step 1: 5 minutes 94°C (denaturation)
Step 2: 3 seconds 94°C
Step 3: 1 minute 56°C (annealing)

Step 4: 2 minutes 72°C (elongation)
30 repeats of steps 2 to 4
Step 5: 10 minutes 72°C (post elongation)
Step 6: 4°C (waiting loop)

In the positively selected plants, the steady-state amount of the mRNA of the codA gene and the amount of CODA protein resulting therefrom is reduced so much that a quantitative conversion of 5-fluorocytosine to 5-fluorouracil can no longer occur. Consequently, these plants (in contrast to the untransformed plants) can grow in the presence of 5-fluorocytosine. Thus it is demonstrated that transgenic plants can be identified owing to the applied principle of preventing expression of a negative selection marker.

Example 9: Detection of the DNA coding for codA-RNAi by using genomic DNA of the positively selected double transgenic plants (A.thaliana [codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT])

The codA-RNAi transgene may be amplified using a codA-specific primer (e.g. codA5'HindIII SEQ ID NO: 50) and a 35S terminator-specific primer (35sT 5' Primer SEQ ID NO: 71). Using this primer combination, it is possible to detect specifically only the DNA coding for the codA RNAi construct, since the codA gene which was already present in the starting plants (A.thaliana [codA]) used for transformation is flanked by the nos terminator.

The PCR conditions to be chosen are as follows:
The PCR was carried out in a 50 µl reaction mixture which contains:

- 2 µl (200ng) of genomic DNA from the A.thaliana [codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT] plants
- 0.2 mM dATP, dTTP, dGTP, dCTP
- 1.5 mM $Mg(OAc)_2$
- 5 µg of bovine serum albumin
- 40 pmol of codA-specific sense primer (SEQ ID NO: 50, 53 or 69)
- 40 pmol of 35sT 5' primer SEQ ID NO: 71
- 15 µl of 3.3x rTth DNA Polymerase XLPuffer (PE Applied Biosystems)
- 5U of rTth DNA Polymerase XL (PE Applied Biosystems)

The PCR was carried out under the following cycle conditions:
Step 1: 5 minutes 94°C (denaturation)

Step 2: 3 seconds 94°C
Step 3: 1 minute 56°C (annealing)
Step 4: 2 minutes 72°C (elongation)
30 repeats of steps 2 to 4
Step 5: 10 minutes 72°C (post elongation)
Step 6: 4°C (waiting loop)

In this way, it is possible to detect in the positively selected plants integration of the codA-RNAi DNA construct into the chromosomal DNA of the starting plants used for transformation. Thus it is demonstrated that transgenic plants can be identified owing to the applied principle of preventing expression of a negative selection marker.

Example 10: Detection of the reduction in the steady-state amount of codA RNA in the positively selected double transgenic plants (A.thaliana [codA]-[codA-RNAi-At.Act.-2-At.Als-R-ocsT]) in comparison with the starting plants (A.thaliana [codA]) used for transformation, by Northern analysis.

Gel-electrophoretic RNA fractionation:

For each RNA agarose gel, 3 g of agar are dissolved in 150 ml of H_2O (f.c. 1.5% (w/v)) in a microwave oven and cooled to 60°C. The addition of 20 ml of 10x MEN (0.2 M MOPS, 50 mM sodium acetate, 10 mM EDTA) and 30 ml of formaldehyde (f.c. 2.2 M) causes further cooling so that the well-mixed solution must be poured speedily. Formaldehyde prevents the formation of secondary structures in the RNA, and therefore the rate of migration is approximately proportional to the molecular weight (LEHRBACH H et al. (1977) Biochem J 16: 4743-4751). The RNA samples are denatured, prior to application to the gel, in the following mixture: 20 µl of RNA (1-2 µg/µl), 5 µl of 10x MEN buffer, 6 µl of formaldehyde, 20 µl of formamide.

The mixture is mixed and incubated at 65°C for 10 minutes. 1/10 volume of sample buffer and 1 µl of ethidium bromide (10 mg/ml) are added and the sample is then applied. Gel electrophoresis is carried out in horizontal gels in 1x MEN at 120 V for two to three hours. After electrophoresis, the gel is photographed under UV light with the aid of a ruler for subsequent determination of the fragment length. This is followed by blotting the RNA to a nylon membrane according to the information in: SAMBROOK J et al. Molecular cloning: A laboratory manual. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, 1989.

Radioactive labeling of DNA fragments and Northern hybridization

The codA cDNA fragment (codARNAi-sense SEQ ID No: 49) can be labeled using, for example, the High Prime kit sold by Roche Diagnostics. The High Prime kit is based on the "random primed" method for DNA labeling originally described by Feinberg and Vogelstein. Labeling is carried out by denaturing approx. 25 ng of DNA in 9-11 µl of H_2O at 95°C for 10 min. After a short incubation on ice, 4 µl of High Prime solution (contains a random primer mixture, 4 units of Klenow polymerase and 0.125 mM dATP, dTTP and dGTP each in a reaction buffer containing 50% glycerol) and 3-5 µl of [α32P]dCTP (30-50 µCi) are added. The reaction mixture is incubated at 37°C for at least 10 min and the unincorporated dCTP is then separated from the now radiolabeled DNA by means of gel filtration via a Sephadex G-50 column. The fragment is subsequently denatured at 95°C for 10 min and kept on ice until used. The following hybridization and preincubation buffers are used:

Hypo Hybond
250 mM sodium phosphate buffer pH 7.2
1 mM EDTA
7% SDS (g/v)
250 mM NaCl
10 µg/ml ssDNA
5% polyethylene glycol (PEG) 6000
40% formamide

The hybridization temperature when using Hypo Hybond is 42°C and the duration of hybridization is 16-24 h. The RNA filters are washed using three different solutions: 2 x SSC (300 mM NaCl; 30 mM sodium citrate) + 0.1% SDS, 1 x SSC + 0,1% SDS and 0.1 x SSC + 0.1% SDS. The duration and intensity of washing depend on the strength of the activity bond. After washing, the filters are sealed in plastic foil and an X-ray film (X-OMat, Kodak) is exposed overnight at -70°C. The signal strength on the X-ray films is a measure of the amount of codA mRNA molecules in the total RNA bound on the membranes. Thus it is possible to detect the reduction in codA mRNA in the positively selected plants compared to the starting plants used for transformation.

In the positively selected plants, the steady-state amount of the mRNA of the codA gene and the amount of CODA protein produced resulting therefrom is reduced so much that a quantitative conversion of 5-fluorocytosine to 5-fluorouracil can no longer occur. Consequently, these plants (in contrast to the untransformed plants) can grow in the presence of 5-fluorocytosine. Thus it is

demonstrated that transgenic plants can be identified owing to the applied principle of preventing expression of a negative selection marker.

Example 11: Summary of the results of "negative-negative" selection

Transformation of the codA-transgenic Arabidopsis plants with the codA-dsRNA construct (pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT; SEQ ID NO: 57) results in a significantly increased number of double transgenic plants into whose genome the RNAi construct has been successfully integrated, in the case of both single selection (with 5-fluorocytosine alone) and dual selection (Pursuit™ and 5-fluorocytosine) (in each case in comparison with untransformed plants). The analysis by means of PCR (see above) confirms the double transgenic state for the majority of the plants generated in this way. This successfully demonstrates the practicability of the present invention, i.e. the usability of repression of a negative marker for positive selection (more or less a "negative-negative" selection).

PF 53790

SEQUENCE LISTING

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Glu Glu Gly Leu Trp Gln Ile His Leu Gln Asp Gly Lys Ile Ser Ala
             20                  25                  30

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gcc gaa caa ggt tta gtt ata ccg ccg ttt gtg gag cca cat att cac   192
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ctg gac acc acg caa acc gcc gga caa ccg aac tgg aat cag tcc ggc   240
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gca acg cta act gcg ctg aaa gca atg ctg gaa gtg aag cag gaa gtc   432
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ggg gca gat gta gtg ggg gcg att ccg cat ttt gaa ttt acc cgt gaa   576
Gly Ala Asp Val Val Gly Ala Ile Pro His Phe Glu Phe Thr Arg Glu
            180                 185                 190

tac ggc gtg gag tcg ctg cat aaa acc ttc gcc ctg gcg caa aaa tac   624
Tyr Gly Val Glu Ser Leu His Lys Thr Phe Ala Leu Ala Gln Lys Tyr
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        35                  40                  45

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                245                 250                 255

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            260                 265                 270

Phe Val Ala Asn Pro Leu Val Asn Ile His Leu Gln Gly Arg Phe Asp
        275                 280                 285

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    290                 295                 300

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Tyr Pro Leu Gly Thr Ala Asn Met Leu Gln Val Leu His Met Gly Leu
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Ile Ala Ala Gly Asn Ser Ala Asn Leu Ile Ile Leu Pro Ala Glu Asn
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Glu Glu Gly Leu Trp Gln Ile His Leu Gln Asp Gly Lys Ile Ser Ala
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gcc gaa caa ggt tta gtt ata ccg ccg ttt gtg gag cca cat att cac   192
Ala Glu Gln Gly Leu Val Ile Pro Pro Phe Val Glu Pro His Ile His
     50                  55                  60

ctg gac acc acg caa acc gcc gga caa ccg aac tgg aat cag tcc ggc   240
Leu Asp Thr Thr Gln Thr Ala Gly Gln Pro Asn Trp Asn Gln Ser Gly
 65                  70                  75                  80

acg ctg ttt gaa ggc att gaa cgc tgg gcc gag cgc aaa gcg tta tta   288
Thr Leu Phe Glu Gly Ile Glu Arg Trp Ala Glu Arg Lys Ala Leu Leu
                 85                  90                  95
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          100                 105                 110

att gcc aac ggc att cag cat gtg cgt acc cat gtc gat gtt tcg gat   384
Ile Ala Asn Gly Ile Gln His Val Arg Thr His Val Asp Val Ser Asp
      115                 120                 125

gca acg cta act gcg ctg aaa gca atg ctg gaa gtg aag cag gaa gtc   432
Ala Thr Leu Thr Ala Leu Lys Ala Met Leu Glu Val Lys Gln Glu Val
  130                 135                 140

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Ala Pro Trp Ile Asp Leu Gln Ile Val Ala Phe Pro Gln Glu Gly Ile
145                 150                 155                 160

ttg tcg tat ccc aac ggt gaa gcg ttg ctg gaa gag gcg tta cgc tta   528
Leu Ser Tyr Pro Asn Gly Glu Ala Leu Leu Glu Glu Ala Leu Arg Leu
                165                 170                 175

ggg gca gat gta gtg ggg gcg att ccg cat ttt gaa ttt acc cgt gaa   576
Gly Ala Asp Val Val Gly Ala Ile Pro His Phe Glu Phe Thr Arg Glu
          180                 185                 190

tac ggc gtg gag tcg ctg cat aaa acc ttc gcc ctg gcg caa aaa tac   624
Tyr Gly Val Glu Ser Leu His Lys Thr Phe Ala Leu Ala Gln Lys Tyr
      195                 200                 205

gac cgt ctc atc gac gtt cac tgt gat gag atc gat gac gag cag tcg   672
Asp Arg Leu Ile Asp Val His Cys Asp Glu Ile Asp Asp Glu Gln Ser
  210                 215                 220

cgc ttt gtc gaa acc gtt gct gcc ctg gcg cac cat gaa ggc atg ggc   720
Arg Phe Val Glu Thr Val Ala Ala Leu Ala His His Glu Gly Met Gly
225                 230                 235                 240

gcg cga gtc acc gcc agc cac acc acg gca atg cac tcc tat aac ggg   768
Ala Arg Val Thr Ala Ser His Thr Thr Ala Met His Ser Tyr Asn Gly
                245                 250                 255

gcg tat acc tca cgc ctg ttc cgc ttg ctg aaa atg tcc ggt att aac   816
Ala Tyr Thr Ser Arg Leu Phe Arg Leu Leu Lys Met Ser Gly Ile Asn
          260                 265                 270

ttt gtc gcc aac ccg ctg gtc aat att cat ctg caa gga cgt ttc gat   864
Phe Val Ala Asn Pro Leu Val Asn Ile His Leu Gln Gly Arg Phe Asp
      275                 280                 285

acg tat cca aaa cgt cgc ggc atc acg cgc gtt aaa gag atg ctg gag   912
Thr Tyr Pro Lys Arg Arg Gly Ile Thr Arg Val Lys Glu Met Leu Glu
  290                 295                 300

tcc ggc att aac gtc tgc ttt ggt cac gat gat gtc ttc gat ccg tgg   960
Ser Gly Ile Asn Val Cys Phe Gly His Asp Asp Val Phe Asp Pro Trp
305                 310                 315                 320

tat ccg ctg gga acg gcg aat atg ctg caa gtg ctg cat atg ggg ctg  1008
Tyr Pro Leu Gly Thr Ala Asn Met Leu Gln Val Leu His Met Gly Leu
                325                 330                 335

cat gtt tgc cag ttg atg ggc tac ggg cag att aac gat ggc ctg aat  1056
His Val Cys Gln Leu Met Gly Tyr Gly Gln Ile Asn Asp Gly Leu Asn
          340                 345                 350

tta atc acc cac cac agc gca agg acg ttg aat ttg cag gat tac ggc  1104
Leu Ile Thr His His Ser Ala Arg Thr Leu Asn Leu Gln Asp Tyr Gly
      355                 360                 365
```

```
att gcc gcc gga aac agc gcc aac ctg att atc ctg ccg gct gaa aat   1152
Ile Ala Ala Gly Asn Ser Ala Asn Leu Ile Ile Leu Pro Ala Glu Asn
    370                 375                 380

ggg ttt gat gcg ctg cgc cgt cag gtt ccg gta cgt tat tcg gta cgt   1200
Gly Phe Asp Ala Leu Arg Arg Gln Val Pro Val Arg Tyr Ser Val Arg
385                 390                 395                 400

ggc ggc aag gtg att gcc agc aca caa ccg gca caa acc acc gta tat   1248
Gly Gly Lys Val Ile Ala Ser Thr Gln Pro Ala Gln Thr Thr Val Tyr
                405                 410                 415

ctg gag cag cca gaa gcc atc gat tac aaa cgt tga                   1284
Leu Glu Gln Pro Glu Ala Ile Asp Tyr Lys Arg
            420                 425
```

<210> 4
<211> 427
<212> PRT
<213> Artificial sequence
<223> Description of the artificial sequence: coding for cytosine deaminase (codA)

<400> 4

```
Met Ser Asn Asn Ala Leu Gln Thr Ile Ile Asn Ala Arg Leu Pro Gly
  1               5                  10                  15

Glu Glu Gly Leu Trp Gln Ile His Leu Gln Asp Gly Lys Ile Ser Ala
            20                  25                  30

Ile Asp Ala Gln Ser Gly Val Met Pro Ile Thr Glu Asn Ser Leu Asp
        35                  40                  45

Ala Glu Gln Gly Leu Val Ile Pro Pro Phe Val Glu Pro His Ile His
    50                  55                  60

Leu Asp Thr Thr Gln Thr Ala Gly Gln Pro Asn Trp Asn Gln Ser Gly
65                  70                  75                  80

Thr Leu Phe Glu Gly Ile Glu Arg Trp Ala Glu Arg Lys Ala Leu Leu
                85                  90                  95

Thr His Asp Asp Val Lys Gln Arg Ala Trp Gln Thr Leu Lys Trp Gln
            100                 105                 110

Ile Ala Asn Gly Ile Gln His Val Arg Thr His Val Asp Val Ser Asp
        115                 120                 125

Ala Thr Leu Thr Ala Leu Lys Ala Met Leu Glu Val Lys Gln Glu Val
    130                 135                 140

Ala Pro Trp Ile Asp Leu Gln Ile Val Ala Phe Pro Gln Glu Gly Ile
145                 150                 155                 160

Leu Ser Tyr Pro Asn Gly Glu Ala Leu Leu Glu Glu Ala Leu Arg Leu
                165                 170                 175

Gly Ala Asp Val Val Gly Ala Ile Pro His Phe Glu Phe Thr Arg Glu
            180                 185                 190

Tyr Gly Val Glu Ser Leu His Lys Thr Phe Ala Leu Ala Gln Lys Tyr
        195                 200                 205

Asp Arg Leu Ile Asp Val His Cys Asp Glu Ile Asp Asp Glu Gln Ser
    210                 215                 220
```

```
Arg Phe Val Glu Thr Val Ala Ala Leu Ala His His Glu Gly Met Gly
225                 230                 235                 240

Ala Arg Val Thr Ala Ser His Thr Thr Ala Met His Ser Tyr Asn Gly
                245                 250                 255

Ala Tyr Thr Ser Arg Leu Phe Arg Leu Leu Lys Met Ser Gly Ile Asn
            260                 265                 270

Phe Val Ala Asn Pro Leu Val Asn Ile His Leu Gln Gly Arg Phe Asp
        275                 280                 285

Thr Tyr Pro Lys Arg Arg Gly Ile Thr Arg Val Lys Glu Met Leu Glu
    290                 295                 300

Ser Gly Ile Asn Val Cys Phe Gly His Asp Asp Val Phe Asp Pro Trp
305                 310                 315                 320

Tyr Pro Leu Gly Thr Ala Asn Met Leu Gln Val Leu His Met Gly Leu
                325                 330                 335

His Val Cys Gln Leu Met Gly Tyr Gly Gln Ile Asn Asp Gly Leu Asn
            340                 345                 350

Leu Ile Thr His His Ser Ala Arg Thr Leu Asn Leu Gln Asp Tyr Gly
        355                 360                 365

Ile Ala Ala Gly Asn Ser Ala Asn Leu Ile Ile Leu Pro Ala Glu Asn
    370                 375                 380

Gly Phe Asp Ala Leu Arg Arg Gln Val Pro Val Arg Tyr Ser Val Arg
385                 390                 395                 400

Gly Gly Lys Val Ile Ala Ser Thr Gln Pro Ala Gln Thr Thr Val Tyr
                405                 410                 415

Leu Glu Gln Pro Glu Ala Ile Asp Tyr Lys Arg
            420                 425
```

<210> 5
<211> 1221
<212> DNA
<213> Streptomyces griseolus

<220>
<221> CDS
<222> (1)..(1218)
<223> coding for cytochrome P450-Su1 (suaC)

<400> 5

```
atg acc gat acc gcc acg acg ccc cag acc acg gac gca ccc gcc ttc   48
Met Thr Asp Thr Ala Thr Thr Pro Gln Thr Thr Asp Ala Pro Ala Phe
  1               5                  10                  15

ccg agc aac cgg agc tgt ccc tac cag tta ccg gac ggc tac gcc cag   96
Pro Ser Asn Arg Ser Cys Pro Tyr Gln Leu Pro Asp Gly Tyr Ala Gln
            20                  25                  30

ctc cgg gac acc ccc ggc ccc ctg cac cgg gtg acg ctc tac gac ggc  144
Leu Arg Asp Thr Pro Gly Pro Leu His Arg Val Thr Leu Tyr Asp Gly
        35                  40                  45

cgt cag gcg tgg gtg gtg acc aag cac gag gcc gcg cgc aaa ctg ctc  192
Arg Gln Ala Trp Val Val Thr Lys His Glu Ala Ala Arg Lys Leu Leu
    50                  55                  60
```

```
ggc gac ccc cgg ctg tcc tcc aac cgg acg gac gac aac ttc ccc gcc   240
Gly Asp Pro Arg Leu Ser Ser Asn Arg Thr Asp Asp Asn Phe Pro Ala
 65                  70                  75                  80

acg tca ccg cgc ttc gag gcc gtc cgg gag agc ccg cag gcg ttc atc   288
Thr Ser Pro Arg Phe Glu Ala Val Arg Glu Ser Pro Gln Ala Phe Ile
                85                  90                  95

ggc ctg gac ccg ccc gag cac ggc acc cgg cgg cgg atg acg atc agc   336
Gly Leu Asp Pro Pro Glu His Gly Thr Arg Arg Arg Met Thr Ile Ser
            100                 105                 110

gag ttc acc gtc aag cgg atc aag ggc atg cgc ccc gag gtc gag gag   384
Glu Phe Thr Val Lys Arg Ile Lys Gly Met Arg Pro Glu Val Glu Glu
        115                 120                 125

gtg gtg cac ggc ttc ctc gac gag atg ctg gcc gcc ggc ccg acc gcc   432
Val Val His Gly Phe Leu Asp Glu Met Leu Ala Ala Gly Pro Thr Ala
    130                 135                 140

gac ctg gtc agt cag ttc gcg ctg ccg gtg ccc tcc atg gtg atc tgc   480
Asp Leu Val Ser Gln Phe Ala Leu Pro Val Pro Ser Met Val Ile Cys
145                 150                 155                 160

cga ctc ctc ggc gtg ccc tac gcc gac cac gag ttc ttc cag gac gcg   528
Arg Leu Leu Gly Val Pro Tyr Ala Asp His Glu Phe Phe Gln Asp Ala
                165                 170                 175

agc aag cgg ctg gtg cag tcc acg gac gcg cag agc gcg ctc acc gcg   576
Ser Lys Arg Leu Val Gln Ser Thr Asp Ala Gln Ser Ala Leu Thr Ala
            180                 185                 190

cgg aac gac ctc gcg ggt tac ctg gac ggc ctc atc acc cag ttc cag   624
Arg Asn Asp Leu Ala Gly Tyr Leu Asp Gly Leu Ile Thr Gln Phe Gln
        195                 200                 205

acc gaa ccg ggc gcg ggc ctg gtg ggc gct ctg gtc gcc gac cag ctg   672
Thr Glu Pro Gly Ala Gly Leu Val Gly Ala Leu Val Ala Asp Gln Leu
    210                 215                 220

gcc aac ggc gag atc gac cgt gag gaa ctg atc tcc acc gcg atg ctg   720
Ala Asn Gly Glu Ile Asp Arg Glu Glu Leu Ile Ser Thr Ala Met Leu
225                 230                 235                 240

ctc ctc atc gcc ggc cac gag acc acg gcc tcg atg acc tcc ctc agc   768
Leu Leu Ile Ala Gly His Glu Thr Thr Ala Ser Met Thr Ser Leu Ser
                245                 250                 255

gtg atc acc ctg ctg gac cac ccc gag cag tac gcc gcc ctg cgc gcc   816
Val Ile Thr Leu Leu Asp His Pro Glu Gln Tyr Ala Ala Leu Arg Ala
            260                 265                 270

gac cgc agc ctc gtg ccc ggc gcg gtg gag gaa ctg ctc cgc tac ctc   864
Asp Arg Ser Leu Val Pro Gly Ala Val Glu Glu Leu Leu Arg Tyr Leu
        275                 280                 285

gcc atc gcc gac atc gcg ggc ggc cgc gtc gcc acg gcg gac atc gag   912
Ala Ile Ala Asp Ile Ala Gly Gly Arg Val Ala Thr Ala Asp Ile Glu
    290                 295                 300

gtc gag ggg cac ctc atc cgg gcc ggc gag ggc gtg atc gtc gtc aac   960
Val Glu Gly His Leu Ile Arg Ala Gly Glu Gly Val Ile Val Val Asn
305                 310                 315                 320

tcg ata gcc aac cgg gac ggc acg gtg tac gag gac ccg gac gcc ctc  1008
Ser Ile Ala Asn Arg Asp Gly Thr Val Tyr Glu Asp Pro Asp Ala Leu
                325                 330                 335
```

```
gac atc cac cgc tcc gcg cgc cac cac ctc gcc ttc ggc ttc ggc gtg   1056
Asp Ile His Arg Ser Ala Arg His His Leu Ala Phe Gly Phe Gly Val
            340                 345                 350

cac cag tgc ctg ggc cag aac ctc gcc cgg ctg gag ctg gag gtc atc   1104
His Gln Cys Leu Gly Gln Asn Leu Ala Arg Leu Glu Leu Glu Val Ile
        355                 360                 365

ctc aac gcc ctc atg gac cgc gtc ccg acg ctg cga ctg gcc gtc ccc   1152
Leu Asn Ala Leu Met Asp Arg Val Pro Thr Leu Arg Leu Ala Val Pro
    370                 375                 380

gtc gag cag ttg gtg ctg cgg ccg ggt acg acg atc cag ggc gtc aac   1200
Val Glu Gln Leu Val Leu Arg Pro Gly Thr Thr Ile Gln Gly Val Asn
385                 390                 395                 400

gaa ctc ccg gtc acc tgg tga                                      1221
Glu Leu Pro Val Thr Trp
                405
```

<210> 6
<211> 406
<212> PRT
<213> Streptomyces griseolus

<400> 6

```
Met Thr Asp Thr Ala Thr Thr Pro Gln Thr Thr Asp Ala Pro Ala Phe
  1               5                  10                  15

Pro Ser Asn Arg Ser Cys Pro Tyr Gln Leu Pro Asp Gly Tyr Ala Gln
            20                  25                  30

Leu Arg Asp Thr Pro Gly Pro Leu His Arg Val Thr Leu Tyr Asp Gly
        35                  40                  45

Arg Gln Ala Trp Val Val Thr Lys His Glu Ala Ala Arg Lys Leu Leu
    50                  55                  60

Gly Asp Pro Arg Leu Ser Ser Asn Arg Thr Asp Asp Asn Phe Pro Ala
65                  70                  75                  80

Thr Ser Pro Arg Phe Glu Ala Val Arg Glu Ser Pro Gln Ala Phe Ile
                85                  90                  95

Gly Leu Asp Pro Pro Glu His Gly Thr Arg Arg Arg Met Thr Ile Ser
            100                 105                 110

Glu Phe Thr Val Lys Arg Ile Lys Gly Met Arg Pro Glu Val Glu Glu
        115                 120                 125

Val Val His Gly Phe Leu Asp Glu Met Leu Ala Ala Gly Pro Thr Ala
    130                 135                 140

Asp Leu Val Ser Gln Phe Ala Leu Pro Val Pro Ser Met Val Ile Cys
145                 150                 155                 160

Arg Leu Leu Gly Val Pro Tyr Ala Asp His Glu Phe Phe Gln Asp Ala
                165                 170                 175

Ser Lys Arg Leu Val Gln Ser Thr Asp Ala Gln Ser Ala Leu Thr Ala
            180                 185                 190

Arg Asn Asp Leu Ala Gly Tyr Leu Asp Gly Leu Ile Thr Gln Phe Gln
        195                 200                 205

Thr Glu Pro Gly Ala Gly Leu Val Gly Ala Leu Val Ala Asp Gln Leu
    210                 215                 220
```

```
Ala Asn Gly Glu Ile Asp Arg Glu Glu Leu Ile Ser Thr Ala Met Leu
225                 230                 235                 240

Leu Leu Ile Ala Gly His Glu Thr Thr Ala Ser Met Thr Ser Leu Ser
                245                 250                 255

Val Ile Thr Leu Leu Asp His Pro Glu Gln Tyr Ala Ala Leu Arg Ala
            260                 265                 270

Asp Arg Ser Leu Val Pro Gly Ala Val Glu Glu Leu Leu Arg Tyr Leu
        275                 280                 285

Ala Ile Ala Asp Ile Ala Gly Gly Arg Val Ala Thr Ala Asp Ile Glu
    290                 295                 300

Val Glu Gly His Leu Ile Arg Ala Gly Glu Gly Val Ile Val Val Asn
305                 310                 315                 320

Ser Ile Ala Asn Arg Asp Gly Thr Val Tyr Glu Asp Pro Asp Ala Leu
                325                 330                 335

Asp Ile His Arg Ser Ala Arg His His Leu Ala Phe Gly Phe Gly Val
            340                 345                 350

His Gln Cys Leu Gly Gln Asn Leu Ala Arg Leu Glu Leu Glu Val Ile
        355                 360                 365

Leu Asn Ala Leu Met Asp Arg Val Pro Thr Leu Arg Leu Ala Val Pro
    370                 375                 380

Val Glu Gln Leu Val Leu Arg Pro Gly Thr Thr Ile Gln Gly Val Asn
385                 390                 395                 400

Glu Leu Pro Val Thr Trp
                405
```

<210> 7
<211> 1404
<212> DNA
<213> Agrobacterium tumefaciens

<220>
<221> CDS
<222> (1)..(1401)
<223> coding for indoleacetamide hydrolase (tms2)

<400> 7

```
atg gtg ccc att acc tcg tta gca caa acc cta gaa cgc ctg aga cgg   48
Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15

aaa gac tac tcc tgc tta gaa cta gta gaa act ctg ata gcg cgt tgc   96
Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
             20                  25                  30

caa gct gca aaa cca tta aat gcc ctt ctg gct aca gac tgg gat ggc  144
Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
         35                  40                  45

ttg cgg cga agc gcc aaa aaa att gat cgt cat gga aac gcc gga tta  192
Leu Arg Arg Ser Ala Lys Lys Ile Asp Arg His Gly Asn Ala Gly Leu
     50                  55                  60

ggt ctt tgc ggc att cca ctc tgt ttt aag gcg aac atc gcg acc ggc  240
Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
 65                  70                  75                  80
```

```
ata ttt cct aca agc gct gct act ccg gcg ctg ata aac cac ttg cca   288
Ile Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
                 85                  90                  95

aag ata cca tcc cgc gtc gca gaa aga ctt ttt tca gct gga gca ctg   336
Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
            100                 105                 110

ccg ggt gcc tcg gga aac atg cat gag tta tcg ttt gga att acg agc   384
Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

aac aac tat gcc acc ggt gcg gtg cgg aac ccg tgg aat cca agt ctg   432
Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

ata cca gga ggc tca agc ggt ggt gtg gct gct gcg gtg gca agc cga   480
Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160

ttg atg tta ggc ggc ata ggc acc gat acc ggt gca tct gtt cgc cta   528
Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

ccc gca gcc ctg tgt ggc gta gta gga ttt cga ccg acg ctt gct cga   576
Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Ala Arg
            180                 185                 190

tat cca aga gat cgg ata ata ccg gtc agc ccc acc cgg gac acc gcc   624
Tyr Pro Arg Asp Arg Ile Ile Pro Val Ser Pro Thr Arg Asp Thr Ala
        195                 200                 205

gga atc ata gcg cag tgc gta gcc gat gtt ata atc ctc gac cag gtg   672
Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
    210                 215                 220

att tcc gga cgg tcg gcg aaa att tca ccc atg ccg ctg aag ggg ctt   720
Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225                 230                 235                 240

cgg atc ggc ctc ccc act acc tac ttt tac gat gac ctt gat gct gat   768
Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
                245                 250                 255

gtg gcc ttc gca gct gaa acg acg att cgc ttg cta gcc aac aga ggc   816
Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
            260                 265                 270

gta acc ttt gtt gaa gcc gac atc ccc cac cta gag gaa ctg aat agt   864
Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
        275                 280                 285

ggg gca agt ttg cca att gcg ctt tac gaa ttt cca cac gct cta aaa   912
Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
    290                 295                 300

aag tat ctc gac gat ttt gtg gga aca gtt tct ttt tct gac gtt atc   960
Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305                 310                 315                 320

aaa gga att cgt agc ccc gat gta gcg aac att gtc agt gcg caa att  1008
Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
                325                 330                 335

gat ggg cat caa att tcc aac gat gaa tat gaa ctg gcg cgt caa tcc  1056
Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
            340                 345                 350
```

```
ttc agg cca agg ctc cag gcc act tat cgg aat tac ttc aga ctc tat   1104
Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
        355                 360                 365

cag tta gat gca atc ctt ttc cca act gca ccc tta gcg gcc aaa gcc   1152
Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
    370                 375                 380

ata ggt cag gag tcg tca gtc atc cac aat ggc tca atg atg aac act   1200
Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Met Asn Thr
385                 390                 395                 400

ttc aag atc tac gtg cga aat gtg gac cca agc agc aac gca ggc cta   1248
Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

cct ggg ttg agc ctt cct gcc tgc ctt aca cct gat cgc ttg cct gtt   1296
Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
            420                 425                 430

gga atg gaa att gat gga tta gcg ggg tca gac cac cgt ctg tta gca   1344
Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
        435                 440                 445

atc ggg gca gca tta gaa aaa gcc ata aat ttt cct tcc ttt ccc gat   1392
Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Pro Ser Phe Pro Asp
    450                 455                 460

gct ttt aat tag                                                  1404
Ala Phe Asn
465
```

<210> 8
<211> 467
<212> PRT
<213> Agrobacterium tumefaciens

<400> 8

```
Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15

Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
            20                  25                  30

Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
        35                  40                  45

Leu Arg Arg Ser Ala Lys Lys Ile Asp Arg His Gly Asn Ala Gly Leu
    50                  55                  60

Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
65                  70                  75                  80

Ile Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
                85                  90                  95

Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
            100                 105                 110

Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160
```

Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
165 170 175

Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Ala Arg
180 185 190

Tyr Pro Arg Asp Arg Ile Ile Pro Val Ser Pro Thr Arg Asp Thr Ala
195 200 205

Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
210 215 220

Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225 230 235 240

Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
245 250 255

Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
260 265 270

Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
275 280 285

Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
290 295 300

Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305 310 315 320

Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
325 330 335

Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
340 345 350

Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
355 360 365

Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
370 375 380

Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Met Asn Thr
385 390 395 400

Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
405 410 415

Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
420 425 430

Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
435 440 445

Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Pro Ser Phe Pro Asp
450 455 460

Ala Phe Asn
465

<210> 9
<211> 1404
<212> DNA
<213> Agrobacterium tumefaciens

<220>
<221> CDS

PF 53790

<222> (1)..(1401)
<223> coding for indoleacetamide hydrolase (tms2)

<400> 9

```
atg gtg ccc att acc tcg tta gca caa acc cta gaa cgc ctg aga cgg   48
Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15

aaa gac tac tcc tgc tta gaa cta gta gaa act ctg ata gcg cgt tgc   96
Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
            20                  25                  30

caa gct gca aaa cca tta aat gcc ctt ctg gct aca gac tgg gat ggc  144
Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
        35                  40                  45

ttg cgg cga agc gcc aaa aaa att gat cgt cat gga aac gcc gga tta  192
Leu Arg Arg Ser Ala Lys Lys Ile Asp Arg His Gly Asn Ala Gly Leu
    50                  55                  60

ggt ctt tgc ggc att cca ctc tgt ttt aag gcg aac atc gcg acc ggc  240
Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
65                  70                  75                  80

ata ttt cct aca agc gct gct act ccg gcg ctg ata aac cac ttg cca  288
Ile Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
                85                  90                  95

aag ata cca tcc cgc gtc gca gaa aga ctt ttt tca gct gga gca ctg  336
Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
            100                 105                 110

ccg ggt gcc tcg gga aac atg cat gag tta tcg ttt gga att acg agc  384
Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

aac aac tat gcc acc ggt gcg gtg cgg aac ccg tgg aat cca agt ctg  432
Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

ata cca gga ggc tca agc ggt ggt gtg gct gct gcg gtg gca agc cga  480
Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160

ttg atg tta ggc ggc ata ggc acc gat acc ggt gca tct gtt cgc cta  528
Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

ccc gca gcc ctg tgt ggc gta gta gga ttt cga ccg acg ctt gct cga  576
Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Ala Arg
            180                 185                 190

tat cca aga gat cgg ata ata ccg gtc agc ccc acc cgg gac acc gcc  624
Tyr Pro Arg Asp Arg Ile Ile Pro Val Ser Pro Thr Arg Asp Thr Ala
        195                 200                 205

gga atc ata gcg cag tgc gta gcc gat gtt ata atc ctc gat cag gtg  672
Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
    210                 215                 220

att tcc gga cgg tcg gcg aaa att tca ccc atg ccg ctg aag ggg ctt  720
Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225                 230                 235                 240

cgg atc ggc ctc ccc act acc tac ttt tac gat gac ctt gat gct gat  768
Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
                245                 250                 255
```

```
gtg gcc ttc gca gct gaa acg acg att cgc ttg cta gcc aac aga ggc   816
Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
            260                 265                 270

gta acc ttt gtt gaa gcc gac atc ccc cac cta gag gaa ctg aat agt   864
Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
        275                 280                 285

ggg gca agt ttg cca att gcg ctt tac gaa ttt cca cac gct cta aaa   912
Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
    290                 295                 300

aag tat ctc gac gat ttt gtg gga aca gtt tct ttt tct gac gtt atc   960
Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305                 310                 315                 320

aaa gga att cgt agc ccc gat gta gcg aac att gtc agt gcg caa att  1008
Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
                325                 330                 335

gat ggg cat caa att tcc aac gat gaa tat gaa ctg gcg cgt caa tcc  1056
Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
            340                 345                 350

ttc agg cca agg ctc cag gcc act tat cgg aat tac ttc aga ctc tat  1104
Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
        355                 360                 365

cag tta gat gca atc ctt ttc cca act gca ccc tta gcg gcc aaa gcc  1152
Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
    370                 375                 380

ata ggt cag gag tcg tca gtc atc cac aat ggc tca atg ata aac act  1200
Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Ile Asn Thr
385                 390                 395                 400

ttc aag atc tac gtg cga aat gtg gac cca agc agc aac gca ggc cta  1248
Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

cct ggg ttg agc ctt cct gcc tgc ctt aca cct gat cgc ttg cct gtt  1296
Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
            420                 425                 430

gga atg gaa att gac gga tta gcg ggg tca gac cac cgt ctg tta gca  1344
Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
        435                 440                 445

atc ggg gca gca tta gaa aaa gcc ata aat ttt cct tcc ttt ccc gat  1392
Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Pro Ser Phe Pro Asp
    450                 455                 460

gct ttt aat tag                                                 1404
Ala Phe Asn
465
```

<210> 10
<211> 467
<212> PRT
<213> Agrobacterium tumefaciens

<400> 10

```
Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15
```

Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
20 25 30

Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
35 40 45

Leu Arg Arg Ser Ala Lys Lys Ile Asp Arg His Gly Asn Ala Gly Leu
50 55 60

Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
65 70 75 80

Ile Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
85 90 95

Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
100 105 110

Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
115 120 125

Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
130 135 140

Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145 150 155 160

Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
165 170 175

Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Ala Arg
180 185 190

Tyr Pro Arg Asp Arg Ile Ile Pro Val Ser Pro Thr Arg Asp Thr Ala
195 200 205

Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
210 215 220

Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225 230 235 240

Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
245 250 255

Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
260 265 270

Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
275 280 285

Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
290 295 300

Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305 310 315 320

Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
325 330 335

Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
340 345 350

Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
355 360 365

Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
370 375 380

```
Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Ile Asn Thr
385                 390                 395                 400

Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
            420                 425                 430

Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
        435                 440                 445

Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Pro Ser Phe Pro Asp
    450                 455                 460

Ala Phe Asn
465
```

<210> 11
<211> 609
<212> DNA
<213> Xanthobacter autotrophicus

<220>
<221> CDS
<222> (1)..(603)
<223> coding for haloalkane dehalogenase

<400> 11

```
atg tca acg ttt ttt gaa ccg gag aac gga atg aaa caa aac gcc aaa   48
Met Ser Thr Phe Phe Glu Pro Glu Asn Gly Met Lys Gln Asn Ala Lys
  1               5                  10                  15

acc gaa cga atc ctg gat gtc gcg ctc gaa ttg ctt gag aca gag ggt   96
Thr Glu Arg Ile Leu Asp Val Ala Leu Glu Leu Leu Glu Thr Glu Gly
            20                  25                  30

gag ttt ggt ttg acg atg agg cag gtg gca acg caa gcg gac atg tcc   144
Glu Phe Gly Leu Thr Met Arg Gln Val Ala Thr Gln Ala Asp Met Ser
        35                  40                  45

ctg agc aac gtt cag tac tat ttc aag tcc gag gac ctg ctc ctc gtg   192
Leu Ser Asn Val Gln Tyr Tyr Phe Lys Ser Glu Asp Leu Leu Leu Val
    50                  55                  60

gcc atg gca gac cgt tac ttt caa cgg tgc ctg aca acc atg gct gag   240
Ala Met Ala Asp Arg Tyr Phe Gln Arg Cys Leu Thr Thr Met Ala Glu
65                  70                  75                  80

cat ccg ccc tta tcg gca ggg cgt gat caa cac gcc cag tta aga gcg   288
His Pro Pro Leu Ser Ala Gly Arg Asp Gln His Ala Gln Leu Arg Ala
                85                  90                  95

ttg tta cga gaa ctg ctc ggt cat ggt ctt gag att tcc gag atg tgt   336
Leu Leu Arg Glu Leu Leu Gly His Gly Leu Glu Ile Ser Glu Met Cys
            100                 105                 110

cga ata ttc agg gag tac tgg gca atc gcc acc cgt aat gaa act gtt   384
Arg Ile Phe Arg Glu Tyr Trp Ala Ile Ala Thr Arg Asn Glu Thr Val
        115                 120                 125

cac ggc tat ctc aag tcg tac tat cgg gat ctc gcc gaa gtg atg gct   432
His Gly Tyr Leu Lys Ser Tyr Tyr Arg Asp Leu Ala Glu Val Met Ala
    130                 135                 140
```

```
gag aag ctt gcg cca ctg gcc agc agc gaa aag gcg ctg gcc gtg gcc    480
Glu Lys Leu Ala Pro Leu Ala Ser Ser Glu Lys Ala Leu Ala Val Ala
145                 150                 155                 160

gta tct ttg gtt att cct tat gtt gag ggg tat tcg gta acg gcc att    528
Val Ser Leu Val Ile Pro Tyr Val Glu Gly Tyr Ser Val Thr Ala Ile
                165                 170                 175

gca atg ccc gaa tcc att gat acg att tcc gag acg ctg acc aat gtg    576
Ala Met Pro Glu Ser Ile Asp Thr Ile Ser Glu Thr Leu Thr Asn Val
            180                 185                 190

gtg ttg gag cag ctt cgc atc agc aat tcatga                        609
Val Leu Glu Gln Leu Arg Ile Ser Asn
        195                 200
```

<210> 12
<211> 201
<212> PRT
<213> Xanthobacter autotrophicus

<400> 12

```
Met Ser Thr Phe Phe Glu Pro Glu Asn Gly Met Lys Gln Asn Ala Lys
  1               5                  10                  15

Thr Glu Arg Ile Leu Asp Val Ala Leu Glu Leu Leu Glu Thr Glu Gly
            20                  25                  30

Glu Phe Gly Leu Thr Met Arg Gln Val Ala Thr Gln Ala Asp Met Ser
        35                  40                  45

Leu Ser Asn Val Gln Tyr Tyr Phe Lys Ser Glu Asp Leu Leu Leu Val
    50                  55                  60

Ala Met Ala Asp Arg Tyr Phe Gln Arg Cys Leu Thr Thr Met Ala Glu
65                  70                  75                  80

His Pro Pro Leu Ser Ala Gly Arg Asp Gln His Ala Gln Leu Arg Ala
                85                  90                  95

Leu Leu Arg Glu Leu Leu Gly His Gly Leu Glu Ile Ser Glu Met Cys
            100                 105                 110

Arg Ile Phe Arg Glu Tyr Trp Ala Ile Ala Thr Arg Asn Glu Thr Val
        115                 120                 125

His Gly Tyr Leu Lys Ser Tyr Tyr Arg Asp Leu Ala Glu Val Met Ala
    130                 135                 140

Glu Lys Leu Ala Pro Leu Ala Ser Ser Glu Lys Ala Leu Ala Val Ala
145                 150                 155                 160

Val Ser Leu Val Ile Pro Tyr Val Glu Gly Tyr Ser Val Thr Ala Ile
                165                 170                 175

Ala Met Pro Glu Ser Ile Asp Thr Ile Ser Glu Thr Leu Thr Asn Val
            180                 185                 190

Val Leu Glu Gln Leu Arg Ile Ser Asn
        195                 200
```

<210> 13
<211> 1131
<212> DNA
<213> Herpes simplex virus 1

PF 53790

<220>
<221> CDS
<222> (1)..(1128)
<223> coding for thymidine kinase (TK)

<400> 13

```
atg gct tcg tac ccc tgc cat caa cac gcg tct gcg ttc gac cag gct   48
Met Ala Ser Tyr Pro Cys His Gln His Ala Ser Ala Phe Asp Gln Ala
  1               5                  10                  15

gcg cgt tct cgc ggc cat agc aac cga cgt acg gcg ttg cgc cct cgc   96
Ala Arg Ser Arg Gly His Ser Asn Arg Arg Thr Ala Leu Arg Pro Arg
             20                  25                  30

cgg cag caa gaa gcc acg gaa gtc cgc ctg gag cag aaa atg ccc acg   144
Arg Gln Gln Glu Ala Thr Glu Val Arg Leu Glu Gln Lys Met Pro Thr
         35                  40                  45

cta ctg cgg gtt tat ata gac ggt cct cac ggg atg ggg aaa acc acc   192
Leu Leu Arg Val Tyr Ile Asp Gly Pro His Gly Met Gly Lys Thr Thr
     50                  55                  60

acc acg caa ctg ctg gtg gcc ctg ggt tcg cgc gac gat atc gtc tac   240
Thr Thr Gln Leu Leu Val Ala Leu Gly Ser Arg Asp Asp Ile Val Tyr
 65                  70                  75                  80

gta ccc gag ccg atg act tac tgg cag gtg ctg ggg gct tcc gag aca   288
Val Pro Glu Pro Met Thr Tyr Trp Gln Val Leu Gly Ala Ser Glu Thr
                 85                  90                  95

atc gcg aac atc tac acc aca caa cac cgc ctc gac cag ggt gag ata   336
Ile Ala Asn Ile Tyr Thr Thr Gln His Arg Leu Asp Gln Gly Glu Ile
            100                 105                 110

tcg gcc ggg gac gcg gcg gtg gta atg aca agc gcc cag ata aca atg   384
Ser Ala Gly Asp Ala Ala Val Val Met Thr Ser Ala Gln Ile Thr Met
        115                 120                 125

ggc atg cct tat gcc gtg acc gac gcc gtt ctg gct cct cat gtc ggg   432
Gly Met Pro Tyr Ala Val Thr Asp Ala Val Leu Ala Pro His Val Gly
    130                 135                 140

ggg gag gct ggg agt tca cat gcc ccg ccc ccg gcc ctc acc ctc atc   480
Gly Glu Ala Gly Ser Ser His Ala Pro Pro Pro Ala Leu Thr Leu Ile
145                 150                 155                 160

ttc gac cgc cat ccc atc gcc gcc ctc ctg tgc tac ccg gcc gcg cga   528
Phe Asp Arg His Pro Ile Ala Ala Leu Leu Cys Tyr Pro Ala Ala Arg
                165                 170                 175

tac ctt atg ggc agc atg acc ccc cag gcc gtg ctg gcg ttc gtg gcc   576
Tyr Leu Met Gly Ser Met Thr Pro Gln Ala Val Leu Ala Phe Val Ala
            180                 185                 190

ctc atc ccg ccg acc ttg ccc ggc aca aac atc gtg ttg ggg gcc ctt   624
Leu Ile Pro Pro Thr Leu Pro Gly Thr Asn Ile Val Leu Gly Ala Leu
        195                 200                 205

ccg gag gac aga cac atc gac cgc ctg gcc aaa cgc cag cgc ccc ggc   672
Pro Glu Asp Arg His Ile Asp Arg Leu Ala Lys Arg Gln Arg Pro Gly
    210                 215                 220

gag cgg ctt gac ctg gct atg ctg gcc gcg att cgc cgc gtt tac ggg   720
Glu Arg Leu Asp Leu Ala Met Leu Ala Ala Ile Arg Arg Val Tyr Gly
225                 230                 235                 240
```

```
ctg ctt gcc aat acg gtg cgg tat ctg cag ggc ggc ggg tcg tgg tgg   768
Leu Leu Ala Asn Thr Val Arg Tyr Leu Gln Gly Gly Gly Ser Trp Trp
                245                 250                 255

gag gat tgg gga cag ctt tcg ggg acg gcc gtg ccg ccc cag ggt gcc   816
Glu Asp Trp Gly Gln Leu Ser Gly Thr Ala Val Pro Pro Gln Gly Ala
            260                 265                 270

gag ccc cag agc aac gcg ggc cca cga ccc cat atc ggg gac acg tta   864
Glu Pro Gln Ser Asn Ala Gly Pro Arg Pro His Ile Gly Asp Thr Leu
        275                 280                 285

ttt acc ctg ttt cgg gcc ccc gag ttg ctg gcc ccc aac ggc gac ctg   912
Phe Thr Leu Phe Arg Ala Pro Glu Leu Leu Ala Pro Asn Gly Asp Leu
    290                 295                 300

tat aac gtg ttt gcc tgg gcc ttg gac gtc ttg gcc aaa cgc ctc cgt   960
Tyr Asn Val Phe Ala Trp Ala Leu Asp Val Leu Ala Lys Arg Leu Arg
305                 310                 315                 320

ccc atg cac gtc ttt atc ctg gat tac gac caa tcg ccc gcc ggc tgc  1008
Pro Met His Val Phe Ile Leu Asp Tyr Asp Gln Ser Pro Ala Gly Cys
                325                 330                 335

cgg gac gcc ctg ctg caa ctt acc tcc ggg atg gtc cag acc cac gtc  1056
Arg Asp Ala Leu Leu Gln Leu Thr Ser Gly Met Val Gln Thr His Val
            340                 345                 350

acc acc cca ggc tcc ata ccg acg atc tgc gac ctg gcg cgc acg ttt  1104
Thr Thr Pro Gly Ser Ile Pro Thr Ile Cys Asp Leu Ala Arg Thr Phe
        355                 360                 365

gcc cgg gag atg ggg gag gct aac tga                             1131
Ala Arg Glu Met Gly Glu Ala Asn
    370                 375
```

<210> 14
<211> 376
<212> PRT
<213> Herpes simplex virus 1

<400> 14

```
Met Ala Ser Tyr Pro Cys His Gln His Ala Ser Ala Phe Asp Gln Ala
  1               5                  10                  15

Ala Arg Ser Arg Gly His Ser Asn Arg Arg Thr Ala Leu Arg Pro Arg
            20                  25                  30

Arg Gln Gln Glu Ala Thr Glu Val Arg Leu Glu Gln Lys Met Pro Thr
        35                  40                  45

Leu Leu Arg Val Tyr Ile Asp Gly Pro His Gly Met Gly Lys Thr Thr
    50                  55                  60

Thr Thr Gln Leu Leu Val Ala Leu Gly Ser Arg Asp Asp Ile Val Tyr
65                  70                  75                  80

Val Pro Glu Pro Met Thr Tyr Trp Gln Val Leu Gly Ala Ser Glu Thr
                85                  90                  95

Ile Ala Asn Ile Tyr Thr Thr Gln His Arg Leu Asp Gln Gly Glu Ile
            100                 105                 110

Ser Ala Gly Asp Ala Ala Val Val Met Thr Ser Ala Gln Ile Thr Met
        115                 120                 125
```

```
Gly Met Pro Tyr Ala Val Thr Asp Ala Val Leu Ala Pro His Val Gly
    130                 135                 140
Gly Glu Ala Gly Ser Ser His Ala Pro Pro Pro Ala Leu Thr Leu Ile
145                 150                 155                 160
Phe Asp Arg His Pro Ile Ala Ala Leu Leu Cys Tyr Pro Ala Ala Arg
                165                 170                 175
Tyr Leu Met Gly Ser Met Thr Pro Gln Ala Val Leu Ala Phe Val Ala
            180                 185                 190
Leu Ile Pro Pro Thr Leu Pro Gly Thr Asn Ile Val Leu Gly Ala Leu
        195                 200                 205
Pro Glu Asp Arg His Ile Asp Arg Leu Ala Lys Arg Gln Arg Pro Gly
    210                 215                 220
Glu Arg Leu Asp Leu Ala Met Leu Ala Ala Ile Arg Arg Val Tyr Gly
225                 230                 235                 240
Leu Leu Ala Asn Thr Val Arg Tyr Leu Gln Gly Gly Gly Ser Trp Trp
                245                 250                 255
Glu Asp Trp Gly Gln Leu Ser Gly Thr Ala Val Pro Pro Gln Gly Ala
            260                 265                 270
Glu Pro Gln Ser Asn Ala Gly Pro Arg Pro His Ile Gly Asp Thr Leu
        275                 280                 285
Phe Thr Leu Phe Arg Ala Pro Glu Leu Leu Ala Pro Asn Gly Asp Leu
    290                 295                 300
Tyr Asn Val Phe Ala Trp Ala Leu Asp Val Leu Ala Lys Arg Leu Arg
305                 310                 315                 320
Pro Met His Val Phe Ile Leu Asp Tyr Asp Gln Ser Pro Ala Gly Cys
                325                 330                 335
Arg Asp Ala Leu Leu Gln Leu Thr Ser Gly Met Val Gln Thr His Val
            340                 345                 350
Thr Thr Pro Gly Ser Ile Pro Thr Ile Cys Asp Leu Ala Arg Thr Phe
        355                 360                 365
Ala Arg Glu Met Gly Glu Ala Asn
    370                 375
```

<210> 15
<211> 1131
<212> DNA
<213> Herpes simplex virus 1

<220>
<221> CDS
<222> (1)..(1128)
<223> coding for thymidine kinase (TK)

<400> 15

```
atg gct tcg tac ccc tgc cat caa cac gcg tct gcg ttc gac cag gct   48
Met Ala Ser Tyr Pro Cys His Gln His Ala Ser Ala Phe Asp Gln Ala
  1               5                  10                  15
gcg cgt tct cgc ggc cat agc aac cga cgt acg gcg ttg cgc cct cgc   96
Ala Arg Ser Arg Gly His Ser Asn Arg Arg Thr Ala Leu Arg Pro Arg
             20                  25                  30
```

```
cgg cag caa gaa gcc acg gaa gtc cgc ctg gag cag aaa atg ccc acg   144
Arg Gln Gln Glu Ala Thr Glu Val Arg Leu Glu Gln Lys Met Pro Thr
          35                  40                  45

cta ctg cgg gtt tat ata gac ggt cct cac ggg atg ggg aaa acc acc   192
Leu Leu Arg Val Tyr Ile Asp Gly Pro His Gly Met Gly Lys Thr Thr
     50                  55                  60

acc acg caa ctg ctg gtg gcc ctg ggt tcg cgc gac gat atc gtc tac   240
Thr Thr Gln Leu Leu Val Ala Leu Gly Ser Arg Asp Asp Ile Val Tyr
 65                  70                  75                  80

gta ccc gag ccg atg act tac tgg cag gtg ctg ggg gct tcc gag aca   288
Val Pro Glu Pro Met Thr Tyr Trp Gln Val Leu Gly Ala Ser Glu Thr
                 85                  90                  95

atc gcg aac atc tac acc aca caa cac cgc ctc gac cag ggt gag ata   336
Ile Ala Asn Ile Tyr Thr Thr Gln His Arg Leu Asp Gln Gly Glu Ile
            100                 105                 110

tcg gcc ggg gac gcg gcg gtg gta atg aca agc gcc cag ata aca atg   384
Ser Ala Gly Asp Ala Ala Val Val Met Thr Ser Ala Gln Ile Thr Met
        115                 120                 125

ggc atg cct tat gcc gtg acc gac gcc gtt ctg gct cct cat gtc ggg   432
Gly Met Pro Tyr Ala Val Thr Asp Ala Val Leu Ala Pro His Val Gly
    130                 135                 140

ggg gag gct ggg agt tca cat gcc ccg ccc ccg gcc ctc acc ctc atc   480
Gly Glu Ala Gly Ser Ser His Ala Pro Pro Pro Ala Leu Thr Leu Ile
145                 150                 155                 160

ttc gac cgc cat ccc atc gcc gcc ctc ctg tgc tac ccg gcc gcg cga   528
Phe Asp Arg His Pro Ile Ala Ala Leu Leu Cys Tyr Pro Ala Ala Arg
                165                 170                 175

tac ctt atg ggc agc atg acc ccc cag gcc gtg ctg gcg ttc gtg gcc   576
Tyr Leu Met Gly Ser Met Thr Pro Gln Ala Val Leu Ala Phe Val Ala
            180                 185                 190

ctc atc ccg ccg acc ttg ccc ggc aca aac atc gtg ttg ggg gcc ctt   624
Leu Ile Pro Pro Thr Leu Pro Gly Thr Asn Ile Val Leu Gly Ala Leu
        195                 200                 205

ccg gag gac aga cac atc gac cgc ctg gcc aaa cgc cag cgc ccc ggc   672
Pro Glu Asp Arg His Ile Asp Arg Leu Ala Lys Arg Gln Arg Pro Gly
    210                 215                 220

gag cgg ctt gac ctg gct atg ctg gcc gcg att cgc cgc gtt tac ggg   720
Glu Arg Leu Asp Leu Ala Met Leu Ala Ala Ile Arg Arg Val Tyr Gly
225                 230                 235                 240

ctg ctt gcc aat acg gtg cgg tat ctg cag ggc ggc ggg tcg tgg tgg   768
Leu Leu Ala Asn Thr Val Arg Tyr Leu Gln Gly Gly Gly Ser Trp Trp
                245                 250                 255

gag gat tgg gga cag ctt tcg ggg acg gcc gtg ccg ccc cag ggt gcc   816
Glu Asp Trp Gly Gln Leu Ser Gly Thr Ala Val Pro Pro Gln Gly Ala
            260                 265                 270

gag ccc cag agc aac gcg ggc cca cga ccc cat atc ggg gac acg tta   864
Glu Pro Gln Ser Asn Ala Gly Pro Arg Pro His Ile Gly Asp Thr Leu
        275                 280                 285

ttt acc ctg ttt cgg gcc ccc gag ttg ctg gcc ccc aac ggc gac ctg   912
Phe Thr Leu Phe Arg Ala Pro Glu Leu Leu Ala Pro Asn Gly Asp Leu
    290                 295                 300
```

```
tat aac gtg ttt gcc tgg gcc ttg gac gtc ttg gcc aaa cgc ctc cgt   960
Tyr Asn Val Phe Ala Trp Ala Leu Asp Val Leu Ala Lys Arg Leu Arg
305                 310                 315                 320

ccc atg cac gtc ttt atc ctg gat tac gac caa tcg ccc gcc ggc tgc   1008
Pro Met His Val Phe Ile Leu Asp Tyr Asp Gln Ser Pro Ala Gly Cys
                325                 330                 335

cgg gac gcc ctg ctg caa ctt acc tcc ggg atg gtc cag acc cac gtc   1056
Arg Asp Ala Leu Leu Gln Leu Thr Ser Gly Met Val Gln Thr His Val
            340                 345                 350

acc acc cca ggc tcc ata ccg acg atc tgc gac ctg gcg cgc acg ttt   1104
Thr Thr Pro Gly Ser Ile Pro Thr Ile Cys Asp Leu Ala Arg Thr Phe
        355                 360                 365

gcc cgg gag atg ggg gag gct aac tga                              1131
Ala Arg Glu Met Gly Glu Ala Asn
    370                 375
```

<210> 16
<211> 376
<212> PRT
<213> Herpes simplex virus 1

<400> 16

```
Met Ala Ser Tyr Pro Cys His Gln His Ala Ser Ala Phe Asp Gln Ala
  1               5                  10                  15

Ala Arg Ser Arg Gly His Ser Asn Arg Arg Thr Ala Leu Arg Pro Arg
            20                  25                  30

Arg Gln Gln Glu Ala Thr Glu Val Arg Leu Glu Gln Lys Met Pro Thr
        35                  40                  45

Leu Leu Arg Val Tyr Ile Asp Gly Pro His Gly Met Gly Lys Thr Thr
    50                  55                  60

Thr Thr Gln Leu Leu Val Ala Leu Gly Ser Arg Asp Asp Ile Val Tyr
65                  70                  75                  80

Val Pro Glu Pro Met Thr Tyr Trp Gln Val Leu Gly Ala Ser Glu Thr
                85                  90                  95

Ile Ala Asn Ile Tyr Thr Thr Gln His Arg Leu Asp Gln Gly Glu Ile
            100                 105                 110

Ser Ala Gly Asp Ala Ala Val Val Met Thr Ser Ala Gln Ile Thr Met
        115                 120                 125

Gly Met Pro Tyr Ala Val Thr Asp Ala Val Leu Ala Pro His Val Gly
    130                 135                 140

Gly Glu Ala Gly Ser Ser His Ala Pro Pro Pro Ala Leu Thr Leu Ile
145                 150                 155                 160

Phe Asp Arg His Pro Ile Ala Ala Leu Leu Cys Tyr Pro Ala Ala Arg
                165                 170                 175

Tyr Leu Met Gly Ser Met Thr Pro Gln Ala Val Leu Ala Phe Val Ala
            180                 185                 190

Leu Ile Pro Pro Thr Leu Pro Gly Thr Asn Ile Val Leu Gly Ala Leu
        195                 200                 205

Pro Glu Asp Arg His Ile Asp Arg Leu Ala Lys Arg Gln Arg Pro Gly
    210                 215                 220
```

```
Glu Arg Leu Asp Leu Ala Met Leu Ala Ala Ile Arg Arg Val Tyr Gly
225                 230                 235                 240

Leu Leu Ala Asn Thr Val Arg Tyr Leu Gln Gly Gly Gly Ser Trp Trp
                245                 250                 255

Glu Asp Trp Gly Gln Leu Ser Gly Thr Ala Val Pro Pro Gln Gly Ala
            260                 265                 270

Glu Pro Gln Ser Asn Ala Gly Pro Arg Pro His Ile Gly Asp Thr Leu
        275                 280                 285

Phe Thr Leu Phe Arg Ala Pro Glu Leu Leu Ala Pro Asn Gly Asp Leu
    290                 295                 300

Tyr Asn Val Phe Ala Trp Ala Leu Asp Val Leu Ala Lys Arg Leu Arg
305                 310                 315                 320

Pro Met His Val Phe Ile Leu Asp Tyr Asp Gln Ser Pro Ala Gly Cys
                325                 330                 335

Arg Asp Ala Leu Leu Gln Leu Thr Ser Gly Met Val Gln Thr His Val
            340                 345                 350

Thr Thr Pro Gly Ser Ile Pro Thr Ile Cys Asp Leu Ala Arg Thr Phe
        355                 360                 365

Ala Arg Glu Met Gly Glu Ala Asn
    370                 375
```

<210> 17
<211> 840
<212> DNA
<213> Toxoplasma gondii

<220>
<221> CDS
<222> (1)..(837)
<223> coding for hypoxanthine-xanthine-guanine
phosphoribosyl transferase (HXGPRTase)

<400> 17

```
atg gcg tcc aaa ccc att gaa gaa tcc cgg tcg caa aaa cgg agt gcc   48
Met Ala Ser Lys Pro Ile Glu Glu Ser Arg Ser Gln Lys Arg Ser Ala
  1               5                  10                  15

ttc tca gac atc ttc tgt tgt tgc act cct aat gaa ggg gct atc gtg   96
Phe Ser Asp Ile Phe Cys Cys Cys Thr Pro Asn Glu Gly Ala Ile Val
             20                  25                  30

ccc agt gac cca atg gtc tcc acc agt gct cca gca cgc acc agt gct   144
Pro Ser Asp Pro Met Val Ser Thr Ser Ala Pro Ala Arg Thr Ser Ala
         35                  40                  45

cca gcg cgc tcc agt gca ctt caa gac tac ggc aag ggc aag ggc cgt   192
Pro Ala Arg Ser Ser Ala Leu Gln Asp Tyr Gly Lys Gly Lys Gly Arg
     50                  55                  60

att gag ccc atg tat atc ccc gac aac acc ttc tac aac gct gat gac   240
Ile Glu Pro Met Tyr Ile Pro Asp Asn Thr Phe Tyr Asn Ala Asp Asp
 65                  70                  75                  80

ttt ctt gtg ccc ccc cac tgc aag ccc tac att gac aaa atc ctc ctc   288
Phe Leu Val Pro Pro His Cys Lys Pro Tyr Ile Asp Lys Ile Leu Leu
                85                  90                  95
```

```
cct ggt gga ttg gtc aag gac aga gtt gag aag ttg gcg tat gac atc   336
Pro Gly Gly Leu Val Lys Asp Arg Val Glu Lys Leu Ala Tyr Asp Ile
            100                 105                 110

cac aga act tac ttc ggc gag gag ttg cac atc att tgc atc ctg aaa   384
His Arg Thr Tyr Phe Gly Glu Glu Leu His Ile Ile Cys Ile Leu Lys
        115                 120                 125

ggc tct cgc ggc ttc ttc aac ctt ctg atc gac tac ctt gcc acc ata   432
Gly Ser Arg Gly Phe Phe Asn Leu Leu Ile Asp Tyr Leu Ala Thr Ile
    130                 135                 140

cag aag tac agt ggt cgt gag tcc agc gtg ccc ccc ttc ttc gag cac   480
Gln Lys Tyr Ser Gly Arg Glu Ser Ser Val Pro Pro Phe Phe Glu His
145                 150                 155                 160

tat gtc cgc ctg aag tcc tac cag aac gac aac agc aca ggc cag ctc   528
Tyr Val Arg Leu Lys Ser Tyr Gln Asn Asp Asn Ser Thr Gly Gln Leu
                165                 170                 175

acc gtc ttg agc gac gac ttg tca atc ttt cgc gac aag cac gtt ctg   576
Thr Val Leu Ser Asp Asp Leu Ser Ile Phe Arg Asp Lys His Val Leu
            180                 185                 190

att gtt gag gac atc gtc gac acc ggt ttc acc ctc acc gag ttc ggt   624
Ile Val Glu Asp Ile Val Asp Thr Gly Phe Thr Leu Thr Glu Phe Gly
        195                 200                 205

gag cgc ctg aaa gcc gtc ggt ccc aag tcg atg aga atc gcc acc ctc   672
Glu Arg Leu Lys Ala Val Gly Pro Lys Ser Met Arg Ile Ala Thr Leu
    210                 215                 220

gtc gag aag cgc aca gat cgc tcc aac agc ttg aag ggc gac ttc gtc   720
Val Glu Lys Arg Thr Asp Arg Ser Asn Ser Leu Lys Gly Asp Phe Val
225                 230                 235                 240

ggc ttc agc att gaa gac gtc tgg atc gtt ggt tgc tgc tac gac ttc   768
Gly Phe Ser Ile Glu Asp Val Trp Ile Val Gly Cys Cys Tyr Asp Phe
                245                 250                 255

aac gag atg ttc cgc gac ttc gac cac gtc gcc gtc ctg agc gac gcc   816
Asn Glu Met Phe Arg Asp Phe Asp His Val Ala Val Leu Ser Asp Ala
            260                 265                 270

gct cgc aaa aag ttc gag aag taa                                  840
Ala Arg Lys Lys Phe Glu Lys
        275
```

<210> 18
<211> 279
<212> PRT
<213> Toxoplasma gondii

<400> 18

```
Met Ala Ser Lys Pro Ile Glu Glu Ser Arg Ser Gln Lys Arg Ser Ala
  1               5                  10                  15

Phe Ser Asp Ile Phe Cys Cys Cys Thr Pro Asn Glu Gly Ala Ile Val
             20                  25                  30

Pro Ser Asp Pro Met Val Ser Thr Ser Ala Pro Ala Arg Thr Ser Ala
         35                  40                  45

Pro Ala Arg Ser Ser Ala Leu Gln Asp Tyr Gly Lys Gly Lys Gly Arg
     50                  55                  60
```

```
Ile Glu Pro Met Tyr Ile Pro Asp Asn Thr Phe Tyr Asn Ala Asp Asp
 65                  70                  75                  80

Phe Leu Val Pro Pro His Cys Lys Pro Tyr Ile Asp Lys Ile Leu Leu
                85                  90                  95

Pro Gly Gly Leu Val Lys Asp Arg Val Glu Lys Leu Ala Tyr Asp Ile
            100                 105                 110

His Arg Thr Tyr Phe Gly Glu Glu Leu His Ile Ile Cys Ile Leu Lys
        115                 120                 125

Gly Ser Arg Gly Phe Phe Asn Leu Leu Ile Asp Tyr Leu Ala Thr Ile
    130                 135                 140

Gln Lys Tyr Ser Gly Arg Glu Ser Ser Val Pro Pro Phe Phe Glu His
145                 150                 155                 160

Tyr Val Arg Leu Lys Ser Tyr Gln Asn Asp Asn Ser Thr Gly Gln Leu
                165                 170                 175

Thr Val Leu Ser Asp Asp Leu Ser Ile Phe Arg Asp Lys His Val Leu
            180                 185                 190

Ile Val Glu Asp Ile Val Asp Thr Gly Phe Thr Leu Thr Glu Phe Gly
        195                 200                 205

Glu Arg Leu Lys Ala Val Gly Pro Lys Ser Met Arg Ile Ala Thr Leu
    210                 215                 220

Val Glu Lys Arg Thr Asp Arg Ser Asn Ser Leu Lys Gly Asp Phe Val
225                 230                 235                 240

Gly Phe Ser Ile Glu Asp Val Trp Ile Val Gly Cys Cys Tyr Asp Phe
                245                 250                 255

Asn Glu Met Phe Arg Asp Phe Asp His Val Ala Val Leu Ser Asp Ala
            260                 265                 270

Ala Arg Lys Lys Phe Glu Lys
        275
```

<210> 19
<211> 459
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(456)
<223> coding for xanthine-guanine phosphoribosyl transferase (gpt)

<400> 19

```
atg agc gaa aaa tac atc gtc acc tgg gac atg ttg cag atc cat gca   48
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
  1               5                  10                  15

cgt aaa ctc gca agc cga ctg atg cct tct gaa caa tgg aaa ggc att   96
Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
             20                  25                  30

att gcc gta agc cgt ggc ggt ctg gta ccg ggt gcg tta ctg gcg cgt   144
Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
         35                  40                  45
```

```
gaa ctg ggt att cgt cat gtc gat acc gtt tgt att tcc agc tac gat   192
Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
     50                  55                  60

cac gac aac cag cgc gag ctt aaa gtg ctg aaa cgc gca gaa ggc gat   240
His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
 65                  70                  75                  80

ggc gaa ggc ttc atc gtt att gat gac ctg gtg gat acc ggt ggt act   288
Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
                 85                  90                  95

gcg gtt gcg att cgt gaa atg tat cca aaa gcg cac ttt gtc acc atc   336
Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
            100                 105                 110

ttc gca aaa ccg gct ggt cgt ccg ctg gtt gat gac tat gtt gtt gat   384
Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
        115                 120                 125

atc ccg caa gat acc tgg att gaa cag ccg tgg gat atg ggc gtc gta   432
Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
    130                 135                 140

ttc gtc ccg cca atc tcc ggt cgc taa                              459
Phe Val Pro Pro Ile Ser Gly Arg
145                 150
```

<210> 20
<211> 152
<212> PRT
<213> Escherichia coli

<400> 20

```
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
  1               5                  10                  15

Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
             20                  25                  30

Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
         35                  40                  45

Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
     50                  55                  60

His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
 65                  70                  75                  80

Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
                 85                  90                  95

Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
            100                 105                 110

Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
        115                 120                 125

Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
    130                 135                 140

Phe Val Pro Pro Ile Ser Gly Arg
145                 150
```

<210> 21
<211> 459
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(456)
<223> coding for xanthine-guanine phosphoribosyl transferase (gpt)

<400> 21

```
atg agc gaa aaa tac atc gtc acc tgg gac atg ttg cag atc cat gca   48
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
  1               5                  10                  15

cgt aaa ctc gca agc cga ctg atg cct tct gaa caa tgg aaa ggc att   96
Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
             20                  25                  30

att gcc gta agc cgt ggc ggt ctg gta ccg ggt gcg tta ctg gcg cgt   144
Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
         35                  40                  45

gaa ctg ggt att cgt cat gtc gat acc gtt tgt att tcc agc tac gat   192
Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
     50                  55                  60

cac gac aac cag cgc gag ctt aaa gtg ctg aaa cgc gca gaa ggc gat   240
His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
 65                  70                  75                  80

ggc gaa ggc ttc atc gtt att gat gac ctg gtg gat acc ggt ggt act   288
Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
                 85                  90                  95

gcg gtt gcg att cgt gaa atg tat cca aaa gcg cac ttt gtc acc atc   336
Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
            100                 105                 110

ttc gca aaa ccg gct ggt cgt ccg ctg gtt gat gac tat gtt gtt gat   384
Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
        115                 120                 125

atc ccg caa gat acc tgg att gaa cag ccg tgg gat atg ggc gtc gta   432
Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
    130                 135                 140

ttc gtc ccg cca atc tcc ggt cgc taa                              459
Phe Val Pro Pro Ile Ser Gly Arg
145                 150
```

<210> 22
<211> 152
<212> PRT
<213> Escherichia coli

<400> 22

```
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
  1               5                  10                  15

Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
             20                  25                  30
```

```
Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
         35                  40                  45
Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
     50                  55                  60
His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
 65                  70                  75                  80
Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
                 85                  90                  95
Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
            100                 105                 110
Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
        115                 120                 125
Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
    130                 135                 140
Phe Val Pro Pro Ile Ser Gly Arg
145                 150
```

<210> 23
<211> 720
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(717)
<223> coding for purine nucleoside phosphorylase (deoD)

<400> 23

```
atg gct acc cca cac att aat gca gaa atg ggc gat ttc gct gac gta   48
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
  1               5                  10                  15
gtt ttg atg cca ggc gac ccg ctg cgt gcg aag tat att gct gaa act   96
Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
             20                  25                  30
ttc ctt gaa gat gcc cgt gaa gtg aac aac gtt cgc ggt atg ctg ggc   144
Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
         35                  40                  45
ttc acc ggt act tac aaa ggc cgc aaa att tcc gta atg ggt cac ggt   192
Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
     50                  55                  60
atg ggt atc ccg tcc tgc tcc atc tac acc aaa gaa ctg atc acc gat   240
Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
 65                  70                  75                  80
ttc ggc gtg aag aaa att atc cgc gtg ggt tcc tgt ggc gca gtt ctg   288
Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
                 85                  90                  95
ccg cac gta aaa ctg cgc gac gtc gtt atc ggt atg ggt gcc tgc acc   336
Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Ala Cys Thr
            100                 105                 110
gat tcc aaa gtt aac cgc atc cgt ttt aaa gac cat gac ttt gcc gct   384
Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125
```

```
atc gct gac ttc gac atg gtg cgt aac gca gta gat gca gct aaa gca   432
Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

ctg ggt att gat gct cgc gtg ggt aac ctg ttc tcc gct gac ctg ttc   480
Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

tac tct ccg gac ggc gaa atg ttc gac gtg atg gaa aaa tac ggc att   528
Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175

ctc ggc gtg gaa atg gaa gcg gct ggt atc tac ggc gtc gct gca gaa   576
Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

ttt ggc gcg aaa gcc ctg acc atc tgc acc gta tct gac cac atc cgc   624
Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

act cac gag cag acc act gcc gct gag cgt cag act acc ttc aac gac   672
Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asp
    210                 215                 220

atg atc aaa atc gca ctg gaa tcc gtt ctg ctg ggc gat aaa gag taa   720
Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 24
<211> 239
<212> PRT
<213> Escherichia coli

<400> 24

```
Met Ala Thr Pro His Ile Asn Ala Glu Met Gly Asp Phe Ala Asp Val
  1               5                  10                  15

Val Leu Met Pro Gly Asp Pro Leu Arg Ala Lys Tyr Ile Ala Glu Thr
            20                  25                  30

Phe Leu Glu Asp Ala Arg Glu Val Asn Asn Val Arg Gly Met Leu Gly
        35                  40                  45

Phe Thr Gly Thr Tyr Lys Gly Arg Lys Ile Ser Val Met Gly His Gly
    50                  55                  60

Met Gly Ile Pro Ser Cys Ser Ile Tyr Thr Lys Glu Leu Ile Thr Asp
65                  70                  75                  80

Phe Gly Val Lys Lys Ile Ile Arg Val Gly Ser Cys Gly Ala Val Leu
                85                  90                  95

Pro His Val Lys Leu Arg Asp Val Val Ile Gly Met Gly Ala Cys Thr
            100                 105                 110

Asp Ser Lys Val Asn Arg Ile Arg Phe Lys Asp His Asp Phe Ala Ala
        115                 120                 125

Ile Ala Asp Phe Asp Met Val Arg Asn Ala Val Asp Ala Ala Lys Ala
    130                 135                 140

Leu Gly Ile Asp Ala Arg Val Gly Asn Leu Phe Ser Ala Asp Leu Phe
145                 150                 155                 160

Tyr Ser Pro Asp Gly Glu Met Phe Asp Val Met Glu Lys Tyr Gly Ile
                165                 170                 175
```

```
Leu Gly Val Glu Met Glu Ala Ala Gly Ile Tyr Gly Val Ala Ala Glu
            180                 185                 190

Phe Gly Ala Lys Ala Leu Thr Ile Cys Thr Val Ser Asp His Ile Arg
        195                 200                 205

Thr His Glu Gln Thr Thr Ala Ala Glu Arg Gln Thr Thr Phe Asn Asp
    210                 215                 220

Met Ile Lys Ile Ala Leu Glu Ser Val Leu Leu Gly Asp Lys Glu
225                 230                 235
```

<210> 25
<211> 1545
<212> DNA
<213> Burkholderia caryophylli

<220>
<221> CDS
<222> (1)..(1542)
<223> coding for phosphonate monoester hydrolase (pehA)

<400> 25

```
atg acc aga aaa aat gtc ctg ctt atc gtc gtt gat caa tgg cga gca   48
Met Thr Arg Lys Asn Val Leu Leu Ile Val Val Asp Gln Trp Arg Ala
  1               5                  10                  15

gat ttt atc cct cac ctg atg cgg gcg gag ggg cgc gaa cct ttc ctt   96
Asp Phe Ile Pro His Leu Met Arg Ala Glu Gly Arg Glu Pro Phe Leu
             20                  25                  30

aaa act ccc aat ctt gat cgt ctt tgc cgg gaa ggc ttg acc ttc cgc   144
Lys Thr Pro Asn Leu Asp Arg Leu Cys Arg Glu Gly Leu Thr Phe Arg
         35                  40                  45

aat cat gtc acg acg tgc gtg ccg tgt ggt ccg gca agg gca agc ctg   192
Asn His Val Thr Thr Cys Val Pro Cys Gly Pro Ala Arg Ala Ser Leu
     50                  55                  60

ctg acg ggc ctc tac ctg atg aac cac cgg gcg gtg cag aac act gtt   240
Leu Thr Gly Leu Tyr Leu Met Asn His Arg Ala Val Gln Asn Thr Val
 65                  70                  75                  80

ccg ctt gac cag cgc cat cta aac ctt ggc aag gcc ctg cgc gcc att   288
Pro Leu Asp Gln Arg His Leu Asn Leu Gly Lys Ala Leu Arg Ala Ile
                 85                  90                  95

ggc tac gat ccc gcg ctc att ggt tac acc acc acg aca cct gat ccg   336
Gly Tyr Asp Pro Ala Leu Ile Gly Tyr Thr Thr Thr Thr Pro Asp Pro
            100                 105                 110

cgc aca acc tct gca agg gat ccg cgt ttc acg gtc ctg ggc gac atc   384
Arg Thr Thr Ser Ala Arg Asp Pro Arg Phe Thr Val Leu Gly Asp Ile
        115                 120                 125

atg gac ggc ttt cgt tcg gtc ggc gca ttc gag ccc aat atg gag ggg   432
Met Asp Gly Phe Arg Ser Val Gly Ala Phe Glu Pro Asn Met Glu Gly
    130                 135                 140

tat ttt ggc tgg gtg gcg cag aac ggc ttc gaa ctg cca gag aac cgc   480
Tyr Phe Gly Trp Val Ala Gln Asn Gly Phe Glu Leu Pro Glu Asn Arg
145                 150                 155                 160
```

```
gaa gat atc tgg ctg ccg gaa ggt gaa cat tcc gtt ccc ggt gct acc   528
Glu Asp Ile Trp Leu Pro Glu Gly Glu His Ser Val Pro Gly Ala Thr
                165                 170                 175

gac aaa ccg tcg cgc att ccg aag gaa ttt tcg gat tcg aca ttc ttc   576
Asp Lys Pro Ser Arg Ile Pro Lys Glu Phe Ser Asp Ser Thr Phe Phe
            180                 185                 190

acg gag cgc gcc ctg aca tat ctg aag ggc agg gac ggc aag cct ttc   624
Thr Glu Arg Ala Leu Thr Tyr Leu Lys Gly Arg Asp Gly Lys Pro Phe
        195                 200                 205

ttc ctg cat ctt ggc tat tat cgc ccg cat ccg cct ttc gta gcc tcc   672
Phe Leu His Leu Gly Tyr Tyr Arg Pro His Pro Pro Phe Val Ala Ser
    210                 215                 220

gcg ccc tac cat gcg atg tac aaa gcc gaa gat atg cct gcg cct ata   720
Ala Pro Tyr His Ala Met Tyr Lys Ala Glu Asp Met Pro Ala Pro Ile
225                 230                 235                 240

cgt gcg gag aat ccg gat gcc gaa gcg gca cag cat ccg ctc atg aag   768
Arg Ala Glu Asn Pro Asp Ala Glu Ala Ala Gln His Pro Leu Met Lys
                245                 250                 255

cac tat atc gac cac atc aga cgc ggc tcg ttc ttc cat ggc gcg gaa   816
His Tyr Ile Asp His Ile Arg Arg Gly Ser Phe Phe His Gly Ala Glu
            260                 265                 270

ggc tcg gga gca acg ctt gat gaa ggc gaa att cgc cag atg cgc gct   864
Gly Ser Gly Ala Thr Leu Asp Glu Gly Glu Ile Arg Gln Met Arg Ala
        275                 280                 285

aca tat tgc gga ctg atc acc gag atc gac gat tgt ctg ggg agg gtc   912
Thr Tyr Cys Gly Leu Ile Thr Glu Ile Asp Asp Cys Leu Gly Arg Val
    290                 295                 300

ttt gcc tat ctc gat gaa acc ggt cag tgg gac gac acg ctg att atc   960
Phe Ala Tyr Leu Asp Glu Thr Gly Gln Trp Asp Asp Thr Leu Ile Ile
305                 310                 315                 320

ttc acg agc gat cat ggc gaa caa ctg ggc gat cat cac ctg ctc ggc  1008
Phe Thr Ser Asp His Gly Glu Gln Leu Gly Asp His His Leu Leu Gly
                325                 330                 335

aag atc ggt tac aat gcc gaa agc ttc cgt att ccc ttg gtc ata aag  1056
Lys Ile Gly Tyr Asn Ala Glu Ser Phe Arg Ile Pro Leu Val Ile Lys
            340                 345                 350

gat gcg gga cag aac cgg cac gcc ggc cag atc gaa gaa ggc ttc tcc  1104
Asp Ala Gly Gln Asn Arg His Ala Gly Gln Ile Glu Glu Gly Phe Ser
        355                 360                 365

gaa agc atc gac gtc atg ccg acc atc ctc gaa tgg ctg ggc ggg gaa  1152
Glu Ser Ile Asp Val Met Pro Thr Ile Leu Glu Trp Leu Gly Gly Glu
    370                 375                 380

acg cct cgc gcc tgc gac ggc cgt tcg ctg ttg ccg ttt ctg gct gag  1200
Thr Pro Arg Ala Cys Asp Gly Arg Ser Leu Leu Pro Phe Leu Ala Glu
385                 390                 395                 400

gga aag ccc tcc gac tgg cgc acg gaa cta cat tac gag ttc gat ttt  1248
Gly Lys Pro Ser Asp Trp Arg Thr Glu Leu His Tyr Glu Phe Asp Phe
                405                 410                 415

cgc gat gtc ttc tac gat cag ccg cag aac tcg gtc cag ctt tcc cag  1296
Arg Asp Val Phe Tyr Asp Gln Pro Gln Asn Ser Val Gln Leu Ser Gln
            420                 425                 430
```

```
gat gat tgc agc ctc tgt gtg atc gag gac gaa aac tac aag tac gtg   1344
Asp Asp Cys Ser Leu Cys Val Ile Glu Asp Glu Asn Tyr Lys Tyr Val
        435                 440                 445

cat ttt gcc gcc ctg ccg ccg ctg ttc ttc gat ctg aag gca gac ccg   1392
His Phe Ala Ala Leu Pro Pro Leu Phe Phe Asp Leu Lys Ala Asp Pro
    450                 455                 460

cat gaa ttc agc aat ctg gct ggc gat cct gct tat gcg gcc ctc gtt   1440
His Glu Phe Ser Asn Leu Ala Gly Asp Pro Ala Tyr Ala Ala Leu Val
465                 470                 475                 480

cgt gac tat gcc cag aag gca ttg tcg tgg cga ctg tct cat gcc gac   1488
Arg Asp Tyr Ala Gln Lys Ala Leu Ser Trp Arg Leu Ser His Ala Asp
                485                 490                 495

cgg aca ctc acc cat tac aga tcc agc ccg caa ggg ctg aca acg cgc   1536
Arg Thr Leu Thr His Tyr Arg Ser Ser Pro Gln Gly Leu Thr Thr Arg
            500                 505                 510

aac cat tga                                                      1545
Asn His
```

<210> 26
<211> 514
<212> PRT
<213> Burkholderia caryophylli

<400> 26

```
Met Thr Arg Lys Asn Val Leu Leu Ile Val Val Asp Gln Trp Arg Ala
  1               5                  10                  15

Asp Phe Ile Pro His Leu Met Arg Ala Glu Gly Arg Glu Pro Phe Leu
            20                  25                  30

Lys Thr Pro Asn Leu Asp Arg Leu Cys Arg Glu Gly Leu Thr Phe Arg
        35                  40                  45

Asn His Val Thr Thr Cys Val Pro Cys Gly Pro Ala Arg Ala Ser Leu
    50                  55                  60

Leu Thr Gly Leu Tyr Leu Met Asn His Arg Ala Val Gln Asn Thr Val
65                  70                  75                  80

Pro Leu Asp Gln Arg His Leu Asn Leu Gly Lys Ala Leu Arg Ala Ile
                85                  90                  95

Gly Tyr Asp Pro Ala Leu Ile Gly Tyr Thr Thr Thr Thr Pro Asp Pro
            100                 105                 110

Arg Thr Thr Ser Ala Arg Asp Pro Arg Phe Thr Val Leu Gly Asp Ile
        115                 120                 125

Met Asp Gly Phe Arg Ser Val Gly Ala Phe Glu Pro Asn Met Glu Gly
    130                 135                 140

Tyr Phe Gly Trp Val Ala Gln Asn Gly Phe Glu Leu Pro Glu Asn Arg
145                 150                 155                 160

Glu Asp Ile Trp Leu Pro Glu Gly Glu His Ser Val Pro Gly Ala Thr
                165                 170                 175

Asp Lys Pro Ser Arg Ile Pro Lys Glu Phe Ser Asp Ser Thr Phe Phe
            180                 185                 190

Thr Glu Arg Ala Leu Thr Tyr Leu Lys Gly Arg Asp Gly Lys Pro Phe
        195                 200                 205
```

Phe Leu His Leu Gly Tyr Tyr Arg Pro His Pro Pro Phe Val Ala Ser
210 215 220

Ala Pro Tyr His Ala Met Tyr Lys Ala Glu Asp Met Pro Ala Pro Ile
225 230 235 240

Arg Ala Glu Asn Pro Asp Ala Glu Ala Ala Gln His Pro Leu Met Lys
245 250 255

His Tyr Ile Asp His Ile Arg Arg Gly Ser Phe Phe His Gly Ala Glu
260 265 270

Gly Ser Gly Ala Thr Leu Asp Glu Gly Glu Ile Arg Gln Met Arg Ala
275 280 285

Thr Tyr Cys Gly Leu Ile Thr Glu Ile Asp Asp Cys Leu Gly Arg Val
290 295 300

Phe Ala Tyr Leu Asp Glu Thr Gly Gln Trp Asp Asp Thr Leu Ile Ile
305 310 315 320

Phe Thr Ser Asp His Gly Glu Gln Leu Gly Asp His His Leu Leu Gly
325 330 335

Lys Ile Gly Tyr Asn Ala Glu Ser Phe Arg Ile Pro Leu Val Ile Lys
340 345 350

Asp Ala Gly Gln Asn Arg His Ala Gly Gln Ile Glu Glu Gly Phe Ser
355 360 365

Glu Ser Ile Asp Val Met Pro Thr Ile Leu Glu Trp Leu Gly Gly Glu
370 375 380

Thr Pro Arg Ala Cys Asp Gly Arg Ser Leu Leu Pro Phe Leu Ala Glu
385 390 395 400

Gly Lys Pro Ser Asp Trp Arg Thr Glu Leu His Tyr Glu Phe Asp Phe
405 410 415

Arg Asp Val Phe Tyr Asp Gln Pro Gln Asn Ser Val Gln Leu Ser Gln
420 425 430

Asp Asp Cys Ser Leu Cys Val Ile Glu Asp Glu Asn Tyr Lys Tyr Val
435 440 445

His Phe Ala Ala Leu Pro Pro Leu Phe Phe Asp Leu Lys Ala Asp Pro
450 455 460

His Glu Phe Ser Asn Leu Ala Gly Asp Pro Ala Tyr Ala Ala Leu Val
465 470 475 480

Arg Asp Tyr Ala Gln Lys Ala Leu Ser Trp Arg Leu Ser His Ala Asp
485 490 495

Arg Thr Leu Thr His Tyr Arg Ser Ser Pro Gln Gly Leu Thr Thr Arg
500 505 510

Asn His

<210> 27
<211> 2250
<212> DNA
<213> Agrobacterium rhizogenes
<220>
<221> CDS

<222> (1)..(2247)
<223> coding for tryptophan oxygenase (aux1)

<400> 27

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atg gct gga tcc tcc ttc aca ttg cca tca act ggc tca gcg ccc ctt   48
Met Ala Gly Ser Ser Phe Thr Leu Pro Ser Thr Gly Ser Ala Pro Leu
  1               5                  10                  15

gat atg atg ctt atc gat gat tca gat ctg ctg caa ttg ggt ctc cag   96
Asp Met Met Leu Ile Asp Asp Ser Asp Leu Leu Gln Leu Gly Leu Gln
            20                  25                  30

cag gta ttc tcg aag cgg tac aca gag aca ccg cag tca cgc tac aaa   144
Gln Val Phe Ser Lys Arg Tyr Thr Glu Thr Pro Gln Ser Arg Tyr Lys
        35                  40                  45

ctg acc agg agg gct tct cca gac gtc tca tct ggc gaa ggc aat gtg   192
Leu Thr Arg Arg Ala Ser Pro Asp Val Ser Ser Gly Glu Gly Asn Val
    50                  55                  60

cat gcc ctt gcg ttc ata tat gtc aac gct gag acg ttg cag atg atc   240
His Ala Leu Ala Phe Ile Tyr Val Asn Ala Glu Thr Leu Gln Met Ile
65                  70                  75                  80

aaa aac gct cga tcg cta acc gaa gcg aac ggc gtc aaa gat ctt gtc   288
Lys Asn Ala Arg Ser Leu Thr Glu Ala Asn Gly Val Lys Asp Leu Val
                85                  90                  95

gcc atc gac gtt ccg cca ttt cga aac gac ttc tca aga gcg cta ctc   336
Ala Ile Asp Val Pro Pro Phe Arg Asn Asp Phe Ser Arg Ala Leu Leu
            100                 105                 110

ctt caa gtg atc aac ttg ttg gga aac aac cga aat gcc gat gac gat   384
Leu Gln Val Ile Asn Leu Leu Gly Asn Asn Arg Asn Ala Asp Asp Asp
        115                 120                 125

ctt agt cac ttc ata gca gtt gct ctc cca aac agc gcc cgc tct aag   432
Leu Ser His Phe Ile Ala Val Ala Leu Pro Asn Ser Ala Arg Ser Lys
    130                 135                 140

atc cta acc acg gca ccg ttc gaa gga agc ttg tca gaa aac ttc agg   480
Ile Leu Thr Thr Ala Pro Phe Glu Gly Ser Leu Ser Glu Asn Phe Arg
145                 150                 155                 160

ggg ttc ccg atc act cgt gaa gga aat gtg gca tgt gaa gtg cta gcc   528
Gly Phe Pro Ile Thr Arg Glu Gly Asn Val Ala Cys Glu Val Leu Ala
                165                 170                 175

tat ggg aat aac ttg atg ccc aag gcc tgc tcc gat tcc ttt cca acc   576
Tyr Gly Asn Asn Leu Met Pro Lys Ala Cys Ser Asp Ser Phe Pro Thr
            180                 185                 190

gtg gat ctt ctt tat gac tat ggc aag ttc ttc gag agt tgc gcg gcc   624
Val Asp Leu Leu Tyr Asp Tyr Gly Lys Phe Phe Glu Ser Cys Ala Ala
        195                 200                 205

gat gga cgt atc ggt tat ttt cct gaa ggc gtt acg aaa cct aaa gtg   672
Asp Gly Arg Ile Gly Tyr Phe Pro Glu Gly Val Thr Lys Pro Lys Val
    210                 215                 220

gct ata att ggc gca ggc ttt tcc ggg ctc gtt gca gcg agc gaa cta   720
Ala Ile Ile Gly Ala Gly Phe Ser Gly Leu Val Ala Ala Ser Glu Leu
225                 230                 235                 240

ctt cat gca ggg gta gac gat gtt acg gtg tat gag gcg agt gat cgg   768
Leu His Ala Gly Val Asp Asp Val Thr Val Tyr Glu Ala Ser Asp Arg
                245                 250                 255
```

```
ctt gga gga aag cta tgg tca cac gga ttt aag agt gct cca aat gtg   816
Leu Gly Gly Lys Leu Trp Ser His Gly Phe Lys Ser Ala Pro Asn Val
            260                 265                 270

ata gcc gag atg ggg gcc atg cgt ttt ccg cga agt gaa tca tgc ttg   864
Ile Ala Glu Met Gly Ala Met Arg Phe Pro Arg Ser Glu Ser Cys Leu
        275                 280                 285

ttc ttc tat ctc aaa aag cac gga ctg gac tcc gtt ggt ctg ttc ccg   912
Phe Phe Tyr Leu Lys Lys His Gly Leu Asp Ser Val Gly Leu Phe Pro
    290                 295                 300

aat ccg gga agt gtc gat acc gca ttg ttc tac agg ggc cgt caa tat   960
Asn Pro Gly Ser Val Asp Thr Ala Leu Phe Tyr Arg Gly Arg Gln Tyr
305                 310                 315                 320

atc tgg aaa gcg gga gag gag cca ccg gag ctg ttt cgt cgt gtg cac  1008
Ile Trp Lys Ala Gly Glu Glu Pro Pro Glu Leu Phe Arg Arg Val His
                325                 330                 335

cat gga tgg cgc gca ttt ttg caa gat ggc tat ctc cat gat gga gtc  1056
His Gly Trp Arg Ala Phe Leu Gln Asp Gly Tyr Leu His Asp Gly Val
            340                 345                 350

atg ttg gcg tca ccg tta gca att gtt gac gcc ttg aat tta ggg cat  1104
Met Leu Ala Ser Pro Leu Ala Ile Val Asp Ala Leu Asn Leu Gly His
        355                 360                 365

cta cag cag gcg cat ggc ttc tgg caa tct tgg ctc aca tat ttt gag  1152
Leu Gln Gln Ala His Gly Phe Trp Gln Ser Trp Leu Thr Tyr Phe Glu
    370                 375                 380

cga gag tct ttc tct tct ggc atc gaa aaa atg ttc ttg ggc aat cat  1200
Arg Glu Ser Phe Ser Ser Gly Ile Glu Lys Met Phe Leu Gly Asn His
385                 390                 395                 400

cct ccg ggg ggt gaa caa tgg aat tcc cta gat gac ttg gat ctt ttc  1248
Pro Pro Gly Gly Glu Gln Trp Asn Ser Leu Asp Asp Leu Asp Leu Phe
                405                 410                 415

aaa gcg ctg ggt att gga tcc ggc gga ttc ggc cct gta ttt gaa agt  1296
Lys Ala Leu Gly Ile Gly Ser Gly Gly Phe Gly Pro Val Phe Glu Ser
            420                 425                 430

ggg ttt atc gag atc ctt cgc tta gtc gtc aac ggg tat gag gat aac  1344
Gly Phe Ile Glu Ile Leu Arg Leu Val Val Asn Gly Tyr Glu Asp Asn
        435                 440                 445

gtg cgg ctg agt tac gaa gga att tct gag ctg cct cat agg atc gcc  1392
Val Arg Leu Ser Tyr Glu Gly Ile Ser Glu Leu Pro His Arg Ile Ala
    450                 455                 460

tca cag gta att aac ggc aga tct att cgc gag cgt aca att cac gtt  1440
Ser Gln Val Ile Asn Gly Arg Ser Ile Arg Glu Arg Thr Ile His Val
465                 470                 475                 480

caa gtc gag cag att gat aga gag gag gat aaa ata aat atc aag atc  1488
Gln Val Glu Gln Ile Asp Arg Glu Glu Asp Lys Ile Asn Ile Lys Ile
                485                 490                 495

aaa gga gga aag gtt gag gtc tat gat cga gta ctg gtt aca tcc ggg  1536
Lys Gly Gly Lys Val Glu Val Tyr Asp Arg Val Leu Val Thr Ser Gly
            500                 505                 510

ttt gcg aac atc gaa atg cgc cat ctc ctg aca tca agc aac gca ttc  1584
Phe Ala Asn Ile Glu Met Arg His Leu Leu Thr Ser Ser Asn Ala Phe
        515                 520                 525
```

```
ttc cat gca gat gta agc cat gca ata ggg aac agt cat atg act ggt   1632
Phe His Ala Asp Val Ser His Ala Ile Gly Asn Ser His Met Thr Gly
    530                 535                 540

gcg tca aaa ctg ttc ttg ctg act aac gaa aaa ttc tgg cta caa cat   1680
Ala Ser Lys Leu Phe Leu Leu Thr Asn Glu Lys Phe Trp Leu Gln His
545                 550                 555                 560

cat ttg cca tcg tgc ata ctc acc acc ggc gtt gca aag gca gtt tat   1728
His Leu Pro Ser Cys Ile Leu Thr Thr Gly Val Ala Lys Ala Val Tyr
                565                 570                 575

tgc tta gac tat gat ccg cga gat cca agc ggc aaa gga ctg gtg ttg   1776
Cys Leu Asp Tyr Asp Pro Arg Asp Pro Ser Gly Lys Gly Leu Val Leu
            580                 585                 590

ata agc tat act tgg gag gat gac tca cat aag ctc cta gcc gtc ccc   1824
Ile Ser Tyr Thr Trp Glu Asp Asp Ser His Lys Leu Leu Ala Val Pro
        595                 600                 605

gac aaa aga gaa agg ttc gca tcg ctg cag cgc gat att ggg agg gca   1872
Asp Lys Arg Glu Arg Phe Ala Ser Leu Gln Arg Asp Ile Gly Arg Ala
    610                 615                 620

ttc cca gat ttt gcc aag cac cta act cct gca gac ggg aac tat gat   1920
Phe Pro Asp Phe Ala Lys His Leu Thr Pro Ala Asp Gly Asn Tyr Asp
625                 630                 635                 640

gat aat atc gtt caa cat gat tgg ctg act gat ccc cac gct ggc gga   1968
Asp Asn Ile Val Gln His Asp Trp Leu Thr Asp Pro His Ala Gly Gly
                645                 650                 655

gcg ttt aaa ctg aac cgc aga ggc aac gac gta tat tca gaa agg ctt   2016
Ala Phe Lys Leu Asn Arg Arg Gly Asn Asp Val Tyr Ser Glu Arg Leu
            660                 665                 670

ttc ttt cag ccc ttt gac gta atg cat ccc gcg gac gat aag gga ctt   2064
Phe Phe Gln Pro Phe Asp Val Met His Pro Ala Asp Asp Lys Gly Leu
        675                 680                 685

tac ttg gcc ggt tgt agc tgt tcc ttc acc gga ggg tgg gtt cat ggt   2112
Tyr Leu Ala Gly Cys Ser Cys Ser Phe Thr Gly Gly Trp Val His Gly
    690                 695                 700

gcc att cag acc gca tgc aac gct acg tgt gcg atc att tat ggt tcc   2160
Ala Ile Gln Thr Ala Cys Asn Ala Thr Cys Ala Ile Ile Tyr Gly Ser
705                 710                 715                 720

gga cac ctg caa gag cta atc cac tgg cga cac ctc aaa gaa ggt aat   2208
Gly His Leu Gln Glu Leu Ile His Trp Arg His Leu Lys Glu Gly Asn
                725                 730                 735

cca ctg gcg cac gct tgg aag cgg tat agg tat caa gcg tga           2250
Pro Leu Ala His Ala Trp Lys Arg Tyr Arg Tyr Gln Ala
            740                 745
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<210> 28
<211> 749
<212> PRT
<213> Agrobacterium rhizogenes

<400> 28

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Met Ala Gly Ser Ser Phe Thr Leu Pro Ser Thr Gly Ser Ala Pro Leu
  1               5                  10                  15
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Asp Met Met Leu Ile Asp Asp Ser Asp Leu Leu Gln Leu Gly Leu Gln
20 25 30

Gln Val Phe Ser Lys Arg Tyr Thr Glu Thr Pro Gln Ser Arg Tyr Lys
35 40 45

Leu Thr Arg Arg Ala Ser Pro Asp Val Ser Ser Gly Glu Gly Asn Val
50 55 60

His Ala Leu Ala Phe Ile Tyr Val Asn Ala Glu Thr Leu Gln Met Ile
65 70 75 80

Lys Asn Ala Arg Ser Leu Thr Glu Ala Asn Gly Val Lys Asp Leu Val
85 90 95

Ala Ile Asp Val Pro Pro Phe Arg Asn Asp Phe Ser Arg Ala Leu Leu
100 105 110

Leu Gln Val Ile Asn Leu Leu Gly Asn Asn Arg Asn Ala Asp Asp Asp
115 120 125

Leu Ser His Phe Ile Ala Val Ala Leu Pro Asn Ser Ala Arg Ser Lys
130 135 140

Ile Leu Thr Thr Ala Pro Phe Glu Gly Ser Leu Ser Glu Asn Phe Arg
145 150 155 160

Gly Phe Pro Ile Thr Arg Glu Gly Asn Val Ala Cys Glu Val Leu Ala
165 170 175

Tyr Gly Asn Asn Leu Met Pro Lys Ala Cys Ser Asp Ser Phe Pro Thr
180 185 190

Val Asp Leu Leu Tyr Asp Tyr Gly Lys Phe Phe Glu Ser Cys Ala Ala
195 200 205

Asp Gly Arg Ile Gly Tyr Phe Pro Glu Gly Val Thr Lys Pro Lys Val
210 215 220

Ala Ile Ile Gly Ala Gly Phe Ser Gly Leu Val Ala Ala Ser Glu Leu
225 230 235 240

Leu His Ala Gly Val Asp Asp Val Thr Val Tyr Glu Ala Ser Asp Arg
245 250 255

Leu Gly Gly Lys Leu Trp Ser His Gly Phe Lys Ser Ala Pro Asn Val
260 265 270

Ile Ala Glu Met Gly Ala Met Arg Phe Pro Arg Ser Glu Ser Cys Leu
275 280 285

Phe Phe Tyr Leu Lys Lys His Gly Leu Asp Ser Val Gly Leu Phe Pro
290 295 300

Asn Pro Gly Ser Val Asp Thr Ala Leu Phe Tyr Arg Gly Arg Gln Tyr
305 310 315 320

Ile Trp Lys Ala Gly Glu Glu Pro Pro Glu Leu Phe Arg Arg Val His
325 330 335

His Gly Trp Arg Ala Phe Leu Gln Asp Gly Tyr Leu His Asp Gly Val
340 345 350

Met Leu Ala Ser Pro Leu Ala Ile Val Asp Ala Leu Asn Leu Gly His
355 360 365

Leu Gln Gln Ala His Gly Phe Trp Gln Ser Trp Leu Thr Tyr Phe Glu
370 375 380

Arg Glu Ser Phe Ser Ser Gly Ile Glu Lys Met Phe Leu Gly Asn His
385 390 395 400

Pro Pro Gly Gly Glu Gln Trp Asn Ser Leu Asp Asp Leu Asp Leu Phe
405 410 415

Lys Ala Leu Gly Ile Gly Ser Gly Gly Phe Gly Pro Val Phe Glu Ser
420 425 430

Gly Phe Ile Glu Ile Leu Arg Leu Val Val Asn Gly Tyr Glu Asp Asn
435 440 445

Val Arg Leu Ser Tyr Glu Gly Ile Ser Glu Leu Pro His Arg Ile Ala
450 455 460

Ser Gln Val Ile Asn Gly Arg Ser Ile Arg Glu Arg Thr Ile His Val
465 470 475 480

Gln Val Glu Gln Ile Asp Arg Glu Glu Asp Lys Ile Asn Ile Lys Ile
485 490 495

Lys Gly Gly Lys Val Glu Val Tyr Asp Arg Val Leu Val Thr Ser Gly
500 505 510

Phe Ala Asn Ile Glu Met Arg His Leu Leu Thr Ser Ser Asn Ala Phe
515 520 525

Phe His Ala Asp Val Ser His Ala Ile Gly Asn Ser His Met Thr Gly
530 535 540

Ala Ser Lys Leu Phe Leu Leu Thr Asn Glu Lys Phe Trp Leu Gln His
545 550 555 560

His Leu Pro Ser Cys Ile Leu Thr Thr Gly Val Ala Lys Ala Val Tyr
565 570 575

Cys Leu Asp Tyr Asp Pro Arg Asp Pro Ser Gly Lys Gly Leu Val Leu
580 585 590

Ile Ser Tyr Thr Trp Glu Asp Asp Ser His Lys Leu Leu Ala Val Pro
595 600 605

Asp Lys Arg Glu Arg Phe Ala Ser Leu Gln Arg Asp Ile Gly Arg Ala
610 615 620

Phe Pro Asp Phe Ala Lys His Leu Thr Pro Ala Asp Gly Asn Tyr Asp
625 630 635 640

Asp Asn Ile Val Gln His Asp Trp Leu Thr Asp Pro His Ala Gly Gly
645 650 655

Ala Phe Lys Leu Asn Arg Arg Gly Asn Asp Val Tyr Ser Glu Arg Leu
660 665 670

Phe Phe Gln Pro Phe Asp Val Met His Pro Ala Asp Asp Lys Gly Leu
675 680 685

Tyr Leu Ala Gly Cys Ser Cys Ser Phe Thr Gly Gly Trp Val His Gly
690 695 700

Ala Ile Gln Thr Ala Cys Asn Ala Thr Cys Ala Ile Ile Tyr Gly Ser
705 710 715 720

Gly His Leu Gln Glu Leu Ile His Trp Arg His Leu Lys Glu Gly Asn
725 730 735

Pro Leu Ala His Ala Trp Lys Arg Tyr Arg Tyr Gln Ala
740 745

<210> 29
<211> 1401
<212> DNA
<213> Agrobacterium rhizogenes

<220>
<221> CDS
<222> (1)..(1398)
<223> coding for indoleacetamide hydrolase

<400> 29

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atg gtg acc ctc tcc tcg atc acc gag acg ctt aaa tgt ctc agg gaa   48
Met Val Thr Leu Ser Ser Ile Thr Glu Thr Leu Lys Cys Leu Arg Glu
  1               5                  10                  15

aga aaa tac tcg tgc ttt gag tta atc gaa acg ata ata gcc cgc tgt   96
Arg Lys Tyr Ser Cys Phe Glu Leu Ile Glu Thr Ile Ile Ala Arg Cys
            20                  25                  30

gaa gca gca aga tcc tta aac gcc ttt ctg gaa acc gac tgg gcg cac   144
Glu Ala Ala Arg Ser Leu Asn Ala Phe Leu Glu Thr Asp Trp Ala His
        35                  40                  45

cta cgg tgg act gcc agc aaa atc gat caa cac gga ggt gcc ggt gtt   192
Leu Arg Trp Thr Ala Ser Lys Ile Asp Gln His Gly Gly Ala Gly Val
    50                  55                  60

ggc cta gct ggc gtt ccc cta tgc ttt aaa gcg aat att gcg aca ggc   240
Gly Leu Ala Gly Val Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
65                  70                  75                  80

agg ttc gcc gcg acc gct ggt acg cca ggc tta cag aac cac aaa ccc   288
Arg Phe Ala Ala Thr Ala Gly Thr Pro Gly Leu Gln Asn His Lys Pro
                85                  90                  95

aag acg cct gcc gga gtt gca cga caa ctt ctc gcg gct ggg gca ctg   336
Lys Thr Pro Ala Gly Val Ala Arg Gln Leu Leu Ala Ala Gly Ala Leu
            100                 105                 110

cct ggc gct tcg gga aac atg cac gaa ttg tct ttt ggg atc acg agc   384
Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

aac aac ttc gcc aca ggc gcc gta cga aac ccg tgg aac cct agt ctc   432
Asn Asn Phe Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

atc cca ggg gga tca agt ggg ggt gtg gcc gcc gcg gtg gcc ggc cga   480
Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Gly Arg
145                 150                 155                 160

ttg atg ctg ggc ggc gtc gga act gac acg gga gcg tcg gtc cgt tta   528
Leu Met Leu Gly Gly Val Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

ccg gcc gcc ttg tgc ggc gtg gtg ggg ttt cgt cct acc gtg ggg cga   576
Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Val Gly Arg
            180                 185                 190

tat cca acg gac gga ata gtt ccg gta agc ccc acc cgg gac acc cct   624
Tyr Pro Thr Asp Gly Ile Val Pro Val Ser Pro Thr Arg Asp Thr Pro
        195                 200                 205
```

```
ggc gtt atc gca cag aat gtt ccg gac gtg att ctt ctt gac ggt atc   672
Gly Val Ile Ala Gln Asn Val Pro Asp Val Ile Leu Leu Asp Gly Ile
    210                 215                 220

att tgc ggg aga ccg ccg gtt aat caa acg gtc cgc ctg aag ggg ctg   720
Ile Cys Gly Arg Pro Pro Val Asn Gln Thr Val Arg Leu Lys Gly Leu
225                 230                 235                 240

cgt ata ggc ttg cca acc gct tac ttt tac aac gac ctg gag ccc gat   768
Arg Ile Gly Leu Pro Thr Ala Tyr Phe Tyr Asn Asp Leu Glu Pro Asp
                245                 250                 255

gtc gcc tta gca gcc gag acg att atc aga gtt ctg gca cgc aaa gat   816
Val Ala Leu Ala Ala Glu Thr Ile Ile Arg Val Leu Ala Arg Lys Asp
            260                 265                 270

gtt act ttt gtt gaa gca gat att cct gat tta gcg cat cac aat gaa   864
Val Thr Phe Val Glu Ala Asp Ile Pro Asp Leu Ala His His Asn Glu
        275                 280                 285

ggg gtc agc ttt ccg act gcc atc tac gaa ttt ccg ttg tcc ctt gaa   912
Gly Val Ser Phe Pro Thr Ala Ile Tyr Glu Phe Pro Leu Ser Leu Glu
    290                 295                 300

cat tat att cag aac ttc gta gag ggt gtt tcc ttt tct gag gtt gtc   960
His Tyr Ile Gln Asn Phe Val Glu Gly Val Ser Phe Ser Glu Val Val
305                 310                 315                 320

aga gcg att cgc agt ccg gat gtt gca agt att ctc aat gca caa ctc  1008
Arg Ala Ile Arg Ser Pro Asp Val Ala Ser Ile Leu Asn Ala Gln Leu
                325                 330                 335

tcg gat aat ctt att tcc aaa agc gag tat tgt ctg gcg cga cgt ttt  1056
Ser Asp Asn Leu Ile Ser Lys Ser Glu Tyr Cys Leu Ala Arg Arg Phe
            340                 345                 350

ttc aga ccg aga ctc caa gcg gcc tac cac agt tac ttc aag gcg cat  1104
Phe Arg Pro Arg Leu Gln Ala Ala Tyr His Ser Tyr Phe Lys Ala His
        355                 360                 365

cag cta gat gca att ctt ttc cca aca gct ccg ttg aca gcc aag cca  1152
Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Thr Ala Lys Pro
    370                 375                 380

att ggc cat gat cta tcg gtg att cac aat ggc tca atg acc gat acc  1200
Ile Gly His Asp Leu Ser Val Ile His Asn Gly Ser Met Thr Asp Thr
385                 390                 395                 400

ttt aaa atc ttc gtg cgg aat gta gat ccc agc agt aat gcg ggc ctg  1248
Phe Lys Ile Phe Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

ccg ggc cta agt ctt ccc gtt tct ctt agt tcc aac ggt ctg cct att  1296
Pro Gly Leu Ser Leu Pro Val Ser Leu Ser Ser Asn Gly Leu Pro Ile
            420                 425                 430

ggc atg gaa atc gat ggc tct gca agc tcg gat gaa cgt ctg tta gca  1344
Gly Met Glu Ile Asp Gly Ser Ala Ser Ser Asp Glu Arg Leu Leu Ala
        435                 440                 445

att gga cta gcg ata gaa gaa gca ata gac ttt agg cat cgt ccg act  1392
Ile Gly Leu Ala Ile Glu Glu Ala Ile Asp Phe Arg His Arg Pro Thr
    450                 455                 460

ctg tcg taa                                                     1401
Leu Ser
465
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<210> 30
<211> 466
<212> PRT
<213> Agrobacterium rhizogenes

<400> 30

Met Val Thr Leu Ser Ser Ile Thr Glu Thr Leu Lys Cys Leu Arg Glu
1 5 10 15

Arg Lys Tyr Ser Cys Phe Glu Leu Ile Glu Thr Ile Ile Ala Arg Cys
20 25 30

Glu Ala Ala Arg Ser Leu Asn Ala Phe Leu Glu Thr Asp Trp Ala His
35 40 45

Leu Arg Trp Thr Ala Ser Lys Ile Asp Gln His Gly Gly Ala Gly Val
50 55 60

Gly Leu Ala Gly Val Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
65 70 75 80

Arg Phe Ala Ala Thr Ala Gly Thr Pro Gly Leu Gln Asn His Lys Pro
85 90 95

Lys Thr Pro Ala Gly Val Ala Arg Gln Leu Leu Ala Ala Gly Ala Leu
100 105 110

Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
115 120 125

Asn Asn Phe Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
130 135 140

Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Gly Arg
145 150 155 160

Leu Met Leu Gly Gly Val Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
165 170 175

Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Val Gly Arg
180 185 190

Tyr Pro Thr Asp Gly Ile Val Pro Val Ser Pro Thr Arg Asp Thr Pro
195 200 205

Gly Val Ile Ala Gln Asn Val Pro Asp Val Ile Leu Leu Asp Gly Ile
210 215 220

Ile Cys Gly Arg Pro Pro Val Asn Gln Thr Val Arg Leu Lys Gly Leu
225 230 235 240

Arg Ile Gly Leu Pro Thr Ala Tyr Phe Tyr Asn Asp Leu Glu Pro Asp
245 250 255

Val Ala Leu Ala Ala Glu Thr Ile Ile Arg Val Leu Ala Arg Lys Asp
260 265 270

Val Thr Phe Val Glu Ala Asp Ile Pro Asp Leu Ala His His Asn Glu
275 280 285

Gly Val Ser Phe Pro Thr Ala Ile Tyr Glu Phe Pro Leu Ser Leu Glu
290 295 300

His Tyr Ile Gln Asn Phe Val Glu Gly Val Ser Phe Ser Glu Val Val
305 310 315 320

Arg Ala Ile Arg Ser Pro Asp Val Ala Ser Ile Leu Asn Ala Gln Leu
325 330 335

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Ser Asp Asn Leu Ile Ser Lys Ser Glu Tyr Cys Leu Ala Arg Arg Phe
            340                 345                 350

Phe Arg Pro Arg Leu Gln Ala Ala Tyr His Ser Tyr Phe Lys Ala His
        355                 360                 365

Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Thr Ala Lys Pro
    370                 375                 380

Ile Gly His Asp Leu Ser Val Ile His Asn Gly Ser Met Thr Asp Thr
385                 390                 395                 400

Phe Lys Ile Phe Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

Pro Gly Leu Ser Leu Pro Val Ser Leu Ser Ser Asn Gly Leu Pro Ile
            420                 425                 430

Gly Met Glu Ile Asp Gly Ser Ala Ser Ser Asp Glu Arg Leu Leu Ala
        435                 440                 445

Ile Gly Leu Ala Ile Glu Glu Ala Ile Asp Phe Arg His Arg Pro Thr
    450                 455                 460

Leu Ser
465
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<210> 31
<211> 2268
<212> DNA
<213> Agrobacterium tumefaciens

<220>
<221> CDS
<222> (1)..(2265)
<223> coding for tryptophan monooxygenase

<400> 31

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atg tca gct tca cct ctc ctt gat aac cag tgc gat cat ttc tct acc   48
Met Ser Ala Ser Pro Leu Leu Asp Asn Gln Cys Asp His Phe Ser Thr
  1               5                  10                  15

aaa atg gtg gat ctg ata atg gtc gat aag gct gat gaa ttg gac cgc   96
Lys Met Val Asp Leu Ile Met Val Asp Lys Ala Asp Glu Leu Asp Arg
             20                  25                  30

agg gtt tcc gat gcc ttc tca gaa cgt gaa gct tct agg gga agg agg   144
Arg Val Ser Asp Ala Phe Ser Glu Arg Glu Ala Ser Arg Gly Arg Arg
         35                  40                  45

att act caa atc tcc ggc gag tgc agc gct ggg tta gct tgc aaa agg   192
Ile Thr Gln Ile Ser Gly Glu Cys Ser Ala Gly Leu Ala Cys Lys Arg
     50                  55                  60

ctg gcc gac ggt cgc ttt ccc gag atc tca act ggt gag aag gta gca   240
Leu Ala Asp Gly Arg Phe Pro Glu Ile Ser Thr Gly Glu Lys Val Ala
 65                  70                  75                  80

gcc ctc tcc gct tac atc tat gtt ggc aag gaa att ctg ggg cgg ata   288
Ala Leu Ser Ala Tyr Ile Tyr Val Gly Lys Glu Ile Leu Gly Arg Ile
                 85                  90                  95

ctt gaa tcg gaa cct tgg gcg cga gca aga gtg agt ggt ctc gtt gcc   336
Leu Glu Ser Glu Pro Trp Ala Arg Ala Arg Val Ser Gly Leu Val Ala
             100                 105                 110
```

```
atc gac ctt gca cca ttt tgt atg gat ttc tcc gaa gca caa ctt ctc   384
Ile Asp Leu Ala Pro Phe Cys Met Asp Phe Ser Glu Ala Gln Leu Leu
        115                 120                 125

caa acc ctg ttt ttg ctg agc ggt aaa aga tgt gca tcc agc gat ctt   432
Gln Thr Leu Phe Leu Leu Ser Gly Lys Arg Cys Ala Ser Ser Asp Leu
    130                 135                 140

agt cat ttc gtg gcc att tca atc tct aag act gcc cgc tcc cga acc   480
Ser His Phe Val Ala Ile Ser Ile Ser Lys Thr Ala Arg Ser Arg Thr
145                 150                 155                 160

ctg caa atg ccg ccg tac gag aaa ggc acg acg aaa cgc gtt acc ggg   528
Leu Gln Met Pro Pro Tyr Glu Lys Gly Thr Thr Lys Arg Val Thr Gly
                165                 170                 175

ttt acc ctg acc ctt gaa gag gcc gta cca ttt gac atg gta gct tat   576
Phe Thr Leu Thr Leu Glu Glu Ala Val Pro Phe Asp Met Val Ala Tyr
            180                 185                 190

ggt cga aac ctg atg ctg aag gct tcg gca ggt tcc ttt cca aca att   624
Gly Arg Asn Leu Met Leu Lys Ala Ser Ala Gly Ser Phe Pro Thr Ile
        195                 200                 205

gac ttg ctc tat gac tac aga tcg ttt ttt gac caa tgt tcc gat att   672
Asp Leu Leu Tyr Asp Tyr Arg Ser Phe Phe Asp Gln Cys Ser Asp Ile
    210                 215                 220

gga cgg atc ggc ttc ttt ccg gaa gat gtt cct aag ccg aaa gtg gcg   720
Gly Arg Ile Gly Phe Phe Pro Glu Asp Val Pro Lys Pro Lys Val Ala
225                 230                 235                 240

atc att ggc gct ggc att tcc gga ctc gtg gta gca agc gaa ctg ctt   768
Ile Ile Gly Ala Gly Ile Ser Gly Leu Val Val Ala Ser Glu Leu Leu
                245                 250                 255

cat gct ggt gta gac gat gtt aca ata tat gaa gca agt gat cgg gtt   816
His Ala Gly Val Asp Asp Val Thr Ile Tyr Glu Ala Ser Asp Arg Val
            260                 265                 270

gga ggc aag ctt tgg tca cat gct ttc aag gat gct ccc agc gtg gtg   864
Gly Gly Lys Leu Trp Ser His Ala Phe Lys Asp Ala Pro Ser Val Val
        275                 280                 285

gcc gaa atg ggg gcg atg cga ttt cct cct gct gca tcg tgc ttg ttt   912
Ala Glu Met Gly Ala Met Arg Phe Pro Pro Ala Ala Ser Cys Leu Phe
    290                 295                 300

ttc ttc ctc gag cgg tac ggc ctg tct tcg atg agg ccg ttc cca aat   960
Phe Phe Leu Glu Arg Tyr Gly Leu Ser Ser Met Arg Pro Phe Pro Asn
305                 310                 315                 320

ccc ggc aca gtc gac act aac ttg gtc tac caa ggc ctc cga tac gtg  1008
Pro Gly Thr Val Asp Thr Asn Leu Val Tyr Gln Gly Leu Arg Tyr Val
                325                 330                 335

tgg aaa gcc ggg cag cag cca ccg aag ctg ttc cat cgc gtt tac agc  1056
Trp Lys Ala Gly Gln Gln Pro Pro Lys Leu Phe His Arg Val Tyr Ser
            340                 345                 350

ggt tgg cgt gcg ttc ttg agg gac ggt ttc cat gag gga gat att gtg  1104
Gly Trp Arg Ala Phe Leu Arg Asp Gly Phe His Glu Gly Asp Ile Val
        355                 360                 365

ttg gct tcg cct gtt gtt att act caa gcc ttg aaa tca gga gac att  1152
Leu Ala Ser Pro Val Val Ile Thr Gln Ala Leu Lys Ser Gly Asp Ile
    370                 375                 380
```

```
agg cgg gct cat gac tcc tgg caa act tgg ctg aac cgt ttc ggg agg   1200
Arg Arg Ala His Asp Ser Trp Gln Thr Trp Leu Asn Arg Phe Gly Arg
385                 390                 395                 400

gag tcc ttc tct tca gcg ata gag agg atc ttt ctg ggc acg cat cct   1248
Glu Ser Phe Ser Ser Ala Ile Glu Arg Ile Phe Leu Gly Thr His Pro
                405                 410                 415

cct ggt ggt gaa aca tgg agt ttc cct cat gat tgg gac cta ttc aag   1296
Pro Gly Gly Glu Thr Trp Ser Phe Pro His Asp Trp Asp Leu Phe Lys
            420                 425                 430

cta atg gga ata gga tct ggc ggg ttt ggt cca gtt ttt gaa agc ggg   1344
Leu Met Gly Ile Gly Ser Gly Gly Phe Gly Pro Val Phe Glu Ser Gly
        435                 440                 445

ttt att gag atc ctt cgc ttg gtc ata aac gga tat gaa gaa aat cag   1392
Phe Ile Glu Ile Leu Arg Leu Val Ile Asn Gly Tyr Glu Glu Asn Gln
    450                 455                 460

cgg atg tgc tct gaa gga atc tca gaa ctt cca cgt cga ata gcc tct   1440
Arg Met Cys Ser Glu Gly Ile Ser Glu Leu Pro Arg Arg Ile Ala Ser
465                 470                 475                 480

caa gtg gtt aac ggt gtg tct gta agc cag cgt ata cgc cat gtt caa   1488
Gln Val Val Asn Gly Val Ser Val Ser Gln Arg Ile Arg His Val Gln
                485                 490                 495

gtc agg gcg att gag aag gaa aag aca aaa ata aag ata agg ctt aag   1536
Val Arg Ala Ile Glu Lys Glu Lys Thr Lys Ile Lys Ile Arg Leu Lys
            500                 505                 510

agc ggg ata tct gaa ctt tat gat aag gtg gtg gtt aca tct gga ctc   1584
Ser Gly Ile Ser Glu Leu Tyr Asp Lys Val Val Val Thr Ser Gly Leu
        515                 520                 525

gca aat atc caa ctc agg cat tgt ctg aca tgc gat acc acc att ttt   1632
Ala Asn Ile Gln Leu Arg His Cys Leu Thr Cys Asp Thr Thr Ile Phe
    530                 535                 540

cgt gca cca gtg aac caa gcg gtt gat aac agc cat atg aca ggc tcg   1680
Arg Ala Pro Val Asn Gln Ala Val Asp Asn Ser His Met Thr Gly Ser
545                 550                 555                 560

tca aaa ctc ttt ctg ctg act gaa cga aaa ttt tgg tta gac cat atc   1728
Ser Lys Leu Phe Leu Leu Thr Glu Arg Lys Phe Trp Leu Asp His Ile
                565                 570                 575

ctc ccg tcc tgt gtc ctc atg gac ggg atc gca aaa gca gtg tac tgc   1776
Leu Pro Ser Cys Val Leu Met Asp Gly Ile Ala Lys Ala Val Tyr Cys
            580                 585                 590

ttg gac tat gag ccg cag gat ccg aat ggt aaa ggt ctg gtg ccc ccc   1824
Leu Asp Tyr Glu Pro Gln Asp Pro Asn Gly Lys Gly Leu Val Pro Pro
        595                 600                 605

act tat aca tgg gag gac gac tcc cac aag ctg ttg gcg gtt ccc gac   1872
Thr Tyr Thr Trp Glu Asp Asp Ser His Lys Leu Leu Ala Val Pro Asp
    610                 615                 620

aaa aaa gag cga ttc tgt ctg ctg cgg gac gca att tcg aga tct ttc   1920
Lys Lys Glu Arg Phe Cys Leu Leu Arg Asp Ala Ile Ser Arg Ser Phe
625                 630                 635                 640

ccg gcg ttt gcc cag cat cta gtt cct gcc tgc gct gat tac gac caa   1968
Pro Ala Phe Ala Gln His Leu Val Pro Ala Cys Ala Asp Tyr Asp Gln
                645                 650                 655
```

```
aat gtt gtt caa cat gat tgg ctt aca gac gag aat gcc ggg gga gct   2016
Asn Val Val Gln His Asp Trp Leu Thr Asp Glu Asn Ala Gly Gly Ala
            660                 665                 670

ttc aaa ctc aac cgg cgt ggc gag gat ttt tat tct gaa gaa ctt ttc   2064
Phe Lys Leu Asn Arg Arg Gly Glu Asp Phe Tyr Ser Glu Glu Leu Phe
        675                 680                 685

ttt caa gcg ctg gac atg cct aat gat acc gga gtt tac ttg gcg ggt   2112
Phe Gln Ala Leu Asp Met Pro Asn Asp Thr Gly Val Tyr Leu Ala Gly
    690                 695                 700

tgc agt tgt tcc ttc acc ggt gga tgg gtg gag ggc gct att cag acc   2160
Cys Ser Cys Ser Phe Thr Gly Gly Trp Val Glu Gly Ala Ile Gln Thr
705                 710                 715                 720

gcg tgt aac gcc gtc tgt gca att atc cac aat tgt gga ggt att ttg   2208
Ala Cys Asn Ala Val Cys Ala Ile Ile His Asn Cys Gly Gly Ile Leu
                725                 730                 735

gca aag gac aat cct ctc gaa cac tct tgg aag aga tat aac tac cgc   2256
Ala Lys Asp Asn Pro Leu Glu His Ser Trp Lys Arg Tyr Asn Tyr Arg
            740                 745                 750

aat aga aat taa                                                  2268
Asn Arg Asn
        755
```

<210> 32
<211> 755
<212> PRT
<213> Agrobacterium tumefaciens

<400> 32

```
Met Ser Ala Ser Pro Leu Leu Asp Asn Gln Cys Asp His Phe Ser Thr
  1               5                  10                  15

Lys Met Val Asp Leu Ile Met Val Asp Lys Ala Asp Glu Leu Asp Arg
            20                  25                  30

Arg Val Ser Asp Ala Phe Ser Glu Arg Glu Ala Ser Arg Gly Arg Arg
        35                  40                  45

Ile Thr Gln Ile Ser Gly Glu Cys Ser Ala Gly Leu Ala Cys Lys Arg
    50                  55                  60

Leu Ala Asp Gly Arg Phe Pro Glu Ile Ser Thr Gly Glu Lys Val Ala
65                  70                  75                  80

Ala Leu Ser Ala Tyr Ile Tyr Val Gly Lys Glu Ile Leu Gly Arg Ile
                85                  90                  95

Leu Glu Ser Glu Pro Trp Ala Arg Ala Arg Val Ser Gly Leu Val Ala
            100                 105                 110

Ile Asp Leu Ala Pro Phe Cys Met Asp Phe Ser Glu Ala Gln Leu Leu
        115                 120                 125

Gln Thr Leu Phe Leu Leu Ser Gly Lys Arg Cys Ala Ser Ser Asp Leu
    130                 135                 140

Ser His Phe Val Ala Ile Ser Ile Ser Lys Thr Ala Arg Ser Arg Thr
145                 150                 155                 160

Leu Gln Met Pro Pro Tyr Glu Lys Gly Thr Thr Lys Arg Val Thr Gly
                165                 170                 175
```

Phe Thr Leu Thr Leu Glu Glu Ala Val Pro Phe Asp Met Val Ala Tyr
180 185 190

Gly Arg Asn Leu Met Leu Lys Ala Ser Ala Gly Ser Phe Pro Thr Ile
195 200 205

Asp Leu Leu Tyr Asp Tyr Arg Ser Phe Phe Asp Gln Cys Ser Asp Ile
210 215 220

Gly Arg Ile Gly Phe Phe Pro Glu Asp Val Pro Lys Pro Lys Val Ala
225 230 235 240

Ile Ile Gly Ala Gly Ile Ser Gly Leu Val Val Ala Ser Glu Leu Leu
245 250 255

His Ala Gly Val Asp Asp Val Thr Ile Tyr Glu Ala Ser Asp Arg Val
260 265 270

Gly Gly Lys Leu Trp Ser His Ala Phe Lys Asp Ala Pro Ser Val Val
275 280 285

Ala Glu Met Gly Ala Met Arg Phe Pro Pro Ala Ala Ser Cys Leu Phe
290 295 300

Phe Phe Leu Glu Arg Tyr Gly Leu Ser Ser Met Arg Pro Phe Pro Asn
305 310 315 320

Pro Gly Thr Val Asp Thr Asn Leu Val Tyr Gln Gly Leu Arg Tyr Val
325 330 335

Trp Lys Ala Gly Gln Gln Pro Pro Lys Leu Phe His Arg Val Tyr Ser
340 345 350

Gly Trp Arg Ala Phe Leu Arg Asp Gly Phe His Glu Gly Asp Ile Val
355 360 365

Leu Ala Ser Pro Val Val Ile Thr Gln Ala Leu Lys Ser Gly Asp Ile
370 375 380

Arg Arg Ala His Asp Ser Trp Gln Thr Trp Leu Asn Arg Phe Gly Arg
385 390 395 400

Glu Ser Phe Ser Ser Ala Ile Glu Arg Ile Phe Leu Gly Thr His Pro
405 410 415

Pro Gly Gly Glu Thr Trp Ser Phe Pro His Asp Trp Asp Leu Phe Lys
420 425 430

Leu Met Gly Ile Gly Ser Gly Gly Phe Gly Pro Val Phe Glu Ser Gly
435 440 445

Phe Ile Glu Ile Leu Arg Leu Val Ile Asn Gly Tyr Glu Glu Asn Gln
450 455 460

Arg Met Cys Ser Glu Gly Ile Ser Glu Leu Pro Arg Arg Ile Ala Ser
465 470 475 480

Gln Val Val Asn Gly Val Ser Val Ser Gln Arg Ile Arg His Val Gln
485 490 495

Val Arg Ala Ile Glu Lys Glu Lys Thr Lys Ile Lys Ile Arg Leu Lys
500 505 510

Ser Gly Ile Ser Glu Leu Tyr Asp Lys Val Val Val Thr Ser Gly Leu
515 520 525

Ala Asn Ile Gln Leu Arg His Cys Leu Thr Cys Asp Thr Thr Ile Phe
530 535 540

```
Arg Ala Pro Val Asn Gln Ala Val Asp Asn Ser His Met Thr Gly Ser
545                 550                 555                 560

Ser Lys Leu Phe Leu Leu Thr Glu Arg Lys Phe Trp Leu Asp His Ile
                565                 570                 575

Leu Pro Ser Cys Val Leu Met Asp Gly Ile Ala Lys Ala Val Tyr Cys
            580                 585                 590

Leu Asp Tyr Glu Pro Gln Asp Pro Asn Gly Lys Gly Leu Val Pro Pro
        595                 600                 605

Thr Tyr Thr Trp Glu Asp Asp Ser His Lys Leu Leu Ala Val Pro Asp
    610                 615                 620

Lys Lys Glu Arg Phe Cys Leu Leu Arg Asp Ala Ile Ser Arg Ser Phe
625                 630                 635                 640

Pro Ala Phe Ala Gln His Leu Val Pro Ala Cys Ala Asp Tyr Asp Gln
                645                 650                 655

Asn Val Val Gln His Asp Trp Leu Thr Asp Glu Asn Ala Gly Gly Ala
            660                 665                 670

Phe Lys Leu Asn Arg Arg Gly Glu Asp Phe Tyr Ser Glu Glu Leu Phe
        675                 680                 685

Phe Gln Ala Leu Asp Met Pro Asn Asp Thr Gly Val Tyr Leu Ala Gly
    690                 695                 700

Cys Ser Cys Ser Phe Thr Gly Gly Trp Val Glu Gly Ala Ile Gln Thr
705                 710                 715                 720

Ala Cys Asn Ala Val Cys Ala Ile Ile His Asn Cys Gly Gly Ile Leu
                725                 730                 735

Ala Lys Asp Asn Pro Leu Glu His Ser Trp Lys Arg Tyr Asn Tyr Arg
            740                 745                 750

Asn Arg Asn
        755
```

<210> 33
<211> 1404
<212> DNA
<213> Agrobacterium tumefaciens

<220>
<221> CDS
<222> (1)..(1401)
<223> coding for indoleacetamide hydrolase

<400> 33

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atg gtg ccc att acc tcg tta gca caa acc cta gaa cgc ctg aga cgg    48
Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15

aaa gac tac tcc tgc tta gaa cta gta gaa act ctg ata gcg cgt tgc    96
Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
             20                  25                  30

caa gct gca aaa cca tta aat gcc ctt ctg gct aca gac tgg gat ggc   144
Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
         35                  40                  45
```

PF 53790


```
ttg cgg cga agc gcc aaa aaa aat gat cgt cat gga aac gcc gga tta   192
Leu Arg Arg Ser Ala Lys Lys Asn Asp Arg His Gly Asn Ala Gly Leu
     50                  55                  60

ggt ctt tgc ggc att cca ctc tgt ttt aag gcg aac atc gcg acc ggc   240
Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
 65                  70                  75                  80

gta ttt cct aca agc gct gct act ccg gcg ctg ata aac cac ttg cca   288
Val Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
                 85                  90                  95

aag ata cca tcc cgc gtc gca gaa aga ctt ttt tca gct gga gca ctg   336
Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
            100                 105                 110

ccg ggt gcc tcg gga aac atg cat gag tta tcg ttt gga att acg agc   384
Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

aac aac tat gcc acc ggt gcg gtg cgg aac ccg tgg aat cca agt ctg   432
Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

ata cca ggg ggt tca agc ggt ggt gtg gct gct gcg gtg gca agc cga   480
Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160

ttg atg tta ggc ggc ata ggc acg gat acc ggt gca tct gtt cgc cta   528
Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

ccg gca gcc ctg tgt ggc gta gta gga ttt cga ccg acg ctt ggt cga   576
Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Gly Arg
            180                 185                 190

tat cca aga gat cgg ata ata ccg ttc agc ccc acc cgg gac acc gcc   624
Tyr Pro Arg Asp Arg Ile Ile Pro Phe Ser Pro Thr Arg Asp Thr Ala
        195                 200                 205

gga atc ata gcg cag tgc gta gcc gat gtt ata atc ctc gac cag gtg   672
Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
    210                 215                 220

att tcc gga cgg tcg gcg aaa att tca ccc atg ccg ctg aag ggg ctt   720
Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225                 230                 235                 240

cgg atc ggc ctc ccc act acc tac ttt tac gat gac ctt gat gct gat   768
Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
                245                 250                 255

gtg gcc ttc gca gct gaa acg acg att cgc ttg cta gcc aac aga ggc   816
Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
            260                 265                 270

gta acc ttt gtt gaa gcc gac atc ccc cac cta gag gaa ttg aac agt   864
Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
        275                 280                 285

ggg gca agt ttg cca att gcg ctt tac gaa ttt cca cac gct cta aaa   912
Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
    290                 295                 300

aag tat ctc gac gat ttt gtg gga aca gtt tct ttt tct gac gtt atc   960
Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305                 310                 315                 320
```

```
aaa gga att cgt agc ccc gat gta gcg aac att gtc agt gcg caa att   1008
Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
                325                 330                 335

gat ggg cat caa att tcc aac gat gaa tat gaa ctg gcg cgt caa tcc   1056
Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
            340                 345                 350

ttc agg cca agg ctc cag gcc act tat cgg aat tac ttc aga ctc tat   1104
Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
        355                 360                 365

cag tta gat gca atc ctt ttc cca act gca ccc tta gcg gcc aaa gcc   1152
Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
    370                 375                 380

ata ggt cag gag tcg tca gtc atc cac aat ggc tca atg atg aac act   1200
Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Met Asn Thr
385                 390                 395                 400

ttc aag atc tac gtg cga aat gtg gac cca agc agc aac gca ggc cta   1248
Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
                405                 410                 415

cct ggg ttg agc ctt cct gcc tgc ctt aca cct gat cgc ttg cct gtt   1296
Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
            420                 425                 430

gga atg gaa att gat gga tta gcg ggg tca gac cac cgt ctg tta gca   1344
Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
        435                 440                 445

atc ggg gca gca tta gaa aaa gct ata aat ttt tct tcc ttt ccc gat   1392
Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Ser Ser Phe Pro Asp
    450                 455                 460

gct ttt aat tag                                                  1404
Ala Phe Asn
465
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<210> 34
<211> 467
<212> PRT
<213> Agrobacterium tumefaciens

<400> 34

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Met Val Pro Ile Thr Ser Leu Ala Gln Thr Leu Glu Arg Leu Arg Arg
  1               5                  10                  15

Lys Asp Tyr Ser Cys Leu Glu Leu Val Glu Thr Leu Ile Ala Arg Cys
             20                  25                  30

Gln Ala Ala Lys Pro Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Gly
         35                  40                  45

Leu Arg Arg Ser Ala Lys Lys Asn Asp Arg His Gly Asn Ala Gly Leu
     50                  55                  60

Gly Leu Cys Gly Ile Pro Leu Cys Phe Lys Ala Asn Ile Ala Thr Gly
 65                  70                  75                  80

Val Phe Pro Thr Ser Ala Ala Thr Pro Ala Leu Ile Asn His Leu Pro
                 85                  90                  95

Lys Ile Pro Ser Arg Val Ala Glu Arg Leu Phe Ser Ala Gly Ala Leu
            100                 105                 110
```

Pro Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
115 120 125

Asn Asn Tyr Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
130 135 140

Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145 150 155 160

Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
165 170 175

Pro Ala Ala Leu Cys Gly Val Val Gly Phe Arg Pro Thr Leu Gly Arg
180 185 190

Tyr Pro Arg Asp Arg Ile Ile Pro Phe Ser Pro Thr Arg Asp Thr Ala
195 200 205

Gly Ile Ile Ala Gln Cys Val Ala Asp Val Ile Ile Leu Asp Gln Val
210 215 220

Ile Ser Gly Arg Ser Ala Lys Ile Ser Pro Met Pro Leu Lys Gly Leu
225 230 235 240

Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
245 250 255

Val Ala Phe Ala Ala Glu Thr Thr Ile Arg Leu Leu Ala Asn Arg Gly
260 265 270

Val Thr Phe Val Glu Ala Asp Ile Pro His Leu Glu Glu Leu Asn Ser
275 280 285

Gly Ala Ser Leu Pro Ile Ala Leu Tyr Glu Phe Pro His Ala Leu Lys
290 295 300

Lys Tyr Leu Asp Asp Phe Val Gly Thr Val Ser Phe Ser Asp Val Ile
305 310 315 320

Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Val Ser Ala Gln Ile
325 330 335

Asp Gly His Gln Ile Ser Asn Asp Glu Tyr Glu Leu Ala Arg Gln Ser
340 345 350

Phe Arg Pro Arg Leu Gln Ala Thr Tyr Arg Asn Tyr Phe Arg Leu Tyr
355 360 365

Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Ala Ala Lys Ala
370 375 380

Ile Gly Gln Glu Ser Ser Val Ile His Asn Gly Ser Met Met Asn Thr
385 390 395 400

Phe Lys Ile Tyr Val Arg Asn Val Asp Pro Ser Ser Asn Ala Gly Leu
405 410 415

Pro Gly Leu Ser Leu Pro Ala Cys Leu Thr Pro Asp Arg Leu Pro Val
420 425 430

Gly Met Glu Ile Asp Gly Leu Ala Gly Ser Asp His Arg Leu Leu Ala
435 440 445

Ile Gly Ala Ala Leu Glu Lys Ala Ile Asn Phe Ser Ser Phe Pro Asp
450 455 460

Ala Phe Asn
465

<210> 35
<211> 1419
<212> DNA
<213> Agrobacterium vitis

<220>
<221> CDS
<222> (1)..(1416)
<223> coding for indoleacetamide hydrolase

<400> 35

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atg gtg acc cta ggt tca atc aag gaa acc ctg gaa tgt ctc agg ctg   48
Met Val Thr Leu Gly Ser Ile Lys Glu Thr Leu Glu Cys Leu Arg Leu
  1               5                  10                  15

aaa aaa tac tcc tgt tcc gaa ctg gct gaa acc ata ata gcc cgt tgc   96
Lys Lys Tyr Ser Cys Ser Glu Leu Ala Glu Thr Ile Ile Ala Arg Cys
            20                  25                  30

gaa gcc gcg aaa tct ctc aat gct ctt ctg gcg act gac tgg gat tac   144
Glu Ala Ala Lys Ser Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Tyr
        35                  40                  45

ctg cgg cgt aat gcc aag aaa gta gat gaa gat gga agc gcc ggc gag   192
Leu Arg Arg Asn Ala Lys Lys Val Asp Glu Asp Gly Ser Ala Gly Glu
    50                  55                  60

ggt ctt gcc ggc atc ccg ctg tgt tct aaa gcg aac att gca aca ggc   240
Gly Leu Ala Gly Ile Pro Leu Cys Ser Lys Ala Asn Ile Ala Thr Gly
65                  70                  75                  80

ata ttc cca gca agc gcg gcc acg ccg gcg ctt gat gaa cat tta cct   288
Ile Phe Pro Ala Ser Ala Ala Thr Pro Ala Leu Asp Glu His Leu Pro
                85                  90                  95

aca aca cca gcc ggc gtc cgt aaa ccg ctt cta gac gct ggg gca ctg   336
Thr Thr Pro Ala Gly Val Arg Lys Pro Leu Leu Asp Ala Gly Ala Leu
            100                 105                 110

ata ggc gct tcg gga aac atg cat gag tta tcg ttt ggc att acc agt   384
Ile Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

aac aac cac gcc act ggt gcg gtg aga aac ccc tgg aat ccc agc tta   432
Asn Asn His Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

ata cca gga ggc tcg agc ggc ggc gtg gct gct gct gta gca tca cgg   480
Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160

tta atg ctc ggc gga att ggc acc gac acg ggg gct tcg gtc cgc cta   528
Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

cct gca tcc cta tgt ggc gta gtg gga ttc cgc ccg acg atc ggc aga   576
Pro Ala Ser Leu Cys Gly Val Val Gly Phe Arg Pro Thr Ile Gly Arg
            180                 185                 190

tat cct gga gac cga att gtg ccg gtt agc ccc acc cgc gat aca gcc   624
Tyr Pro Gly Asp Arg Ile Val Pro Val Ser Pro Thr Arg Asp Thr Ala
        195                 200                 205
```

```
gga att atc gca cag agc gtt cct gat gtg ata ctc ctt gac caa atc   672
Gly Ile Ile Ala Gln Ser Val Pro Asp Val Ile Leu Leu Asp Gln Ile
    210                 215                 220

att tgc ggg aag ctc acg acc cac caa cct gta ccc ctg gag gga tta   720
Ile Cys Gly Lys Leu Thr Thr His Gln Pro Val Pro Leu Glu Gly Leu
225                 230                 235                 240

cgt atc ggc ttg cca acc act tac ttt tac gat gac ctt gat gct gat   768
Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
                245                 250                 255

gtg gcc ttc gca gct gaa aac ctt atc acg ctg ctg gcc agc aag ggt   816
Val Ala Phe Ala Ala Glu Asn Leu Ile Thr Leu Leu Ala Ser Lys Gly
            260                 265                 270

gta acc ttt gtt aag gcc gag att cca gat ctg cag cgt ctg aac atc   864
Val Thr Phe Val Lys Ala Glu Ile Pro Asp Leu Gln Arg Leu Asn Ile
        275                 280                 285

ggg gtt agc ttt cct att gcc ctg tac gag ttt ccg ttc gcc cta caa   912
Gly Val Ser Phe Pro Ile Ala Leu Tyr Glu Phe Pro Phe Ala Leu Gln
    290                 295                 300

aag tat atc gat gac ttt gtg aag gat gtg tct ttt tct gac gtc atc   960
Lys Tyr Ile Asp Asp Phe Val Lys Asp Val Ser Phe Ser Asp Val Ile
305                 310                 315                 320

aaa gga att cgt agc cct gat gta gcc aac att gcc aat gct caa att  1008
Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Ala Asn Ala Gln Ile
                325                 330                 335

gat gga cat caa att tcc aaa gct tca tat gaa ctg gcg cga caa tct  1056
Asp Gly His Gln Ile Ser Lys Ala Ser Tyr Glu Leu Ala Arg Gln Ser
            340                 345                 350

ttc aga cca aag ctg caa gcc gcc tac cat gat tac ttc aag ctg cac  1104
Phe Arg Pro Lys Leu Gln Ala Ala Tyr His Asp Tyr Phe Lys Leu His
        355                 360                 365

cag cta gac gcg atc ctt ttc ccg aca gct ccc ctg aca gcc aaa ccg  1152
Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Thr Ala Lys Pro
    370                 375                 380

atc ggc caa gat tta tcg gtg atg cac aat ggc gta atg gcc gac acg  1200
Ile Gly Gln Asp Leu Ser Val Met His Asn Gly Val Met Ala Asp Thr
385                 390                 395                 400

ttt aaa atc ttc gtg cga aat gtg gat ccg ggg agc aac gca ggc ctg  1248
Phe Lys Ile Phe Val Arg Asn Val Asp Pro Gly Ser Asn Ala Gly Leu
                405                 410                 415

cca gga tta agc ctt ccc gtt tct ctt act tca aag ggt ttg cct att  1296
Pro Gly Leu Ser Leu Pro Val Ser Leu Thr Ser Lys Gly Leu Pro Ile
            420                 425                 430

gga atg gaa atc gat gga tta gcg ggc atg gac gac cgt ttg cta gca  1344
Gly Met Glu Ile Asp Gly Leu Ala Gly Met Asp Asp Arg Leu Leu Ala
        435                 440                 445

atc gga gcg gca cta gag gaa gcg ata gct ttt cat aat tta cct gac  1392
Ile Gly Ala Ala Leu Glu Glu Ala Ile Ala Phe His Asn Leu Pro Asp
    450                 455                 460

ttc ccg aaa gtc gag aca aac tac tga                             1419
Phe Pro Lys Val Glu Thr Asn Tyr
465                 470
```

<210> 36
<211> 472
<212> PRT
<213> Agrobacterium vitis

<400> 36

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Met Val Thr Leu Gly Ser Ile Lys Glu Thr Leu Glu Cys Leu Arg Leu
  1               5                  10                  15

Lys Lys Tyr Ser Cys Ser Glu Leu Ala Glu Thr Ile Ile Ala Arg Cys
            20                  25                  30

Glu Ala Ala Lys Ser Leu Asn Ala Leu Leu Ala Thr Asp Trp Asp Tyr
        35                  40                  45

Leu Arg Arg Asn Ala Lys Lys Val Asp Glu Asp Gly Ser Ala Gly Glu
    50                  55                  60

Gly Leu Ala Gly Ile Pro Leu Cys Ser Lys Ala Asn Ile Ala Thr Gly
65                  70                  75                  80

Ile Phe Pro Ala Ser Ala Ala Thr Pro Ala Leu Asp Glu His Leu Pro
                85                  90                  95

Thr Thr Pro Ala Gly Val Arg Lys Pro Leu Leu Asp Ala Gly Ala Leu
            100                 105                 110

Ile Gly Ala Ser Gly Asn Met His Glu Leu Ser Phe Gly Ile Thr Ser
        115                 120                 125

Asn Asn His Ala Thr Gly Ala Val Arg Asn Pro Trp Asn Pro Ser Leu
    130                 135                 140

Ile Pro Gly Gly Ser Ser Gly Gly Val Ala Ala Ala Val Ala Ser Arg
145                 150                 155                 160

Leu Met Leu Gly Gly Ile Gly Thr Asp Thr Gly Ala Ser Val Arg Leu
                165                 170                 175

Pro Ala Ser Leu Cys Gly Val Val Gly Phe Arg Pro Thr Ile Gly Arg
            180                 185                 190

Tyr Pro Gly Asp Arg Ile Val Pro Val Ser Pro Thr Arg Asp Thr Ala
        195                 200                 205

Gly Ile Ile Ala Gln Ser Val Pro Asp Val Ile Leu Leu Asp Gln Ile
    210                 215                 220

Ile Cys Gly Lys Leu Thr Thr His Gln Pro Val Pro Leu Glu Gly Leu
225                 230                 235                 240

Arg Ile Gly Leu Pro Thr Thr Tyr Phe Tyr Asp Asp Leu Asp Ala Asp
                245                 250                 255

Val Ala Phe Ala Ala Glu Asn Leu Ile Thr Leu Leu Ala Ser Lys Gly
            260                 265                 270

Val Thr Phe Val Lys Ala Glu Ile Pro Asp Leu Gln Arg Leu Asn Ile
        275                 280                 285

Gly Val Ser Phe Pro Ile Ala Leu Tyr Glu Phe Pro Phe Ala Leu Gln
    290                 295                 300

Lys Tyr Ile Asp Asp Phe Val Lys Asp Val Ser Phe Ser Asp Val Ile
305                 310                 315                 320

Lys Gly Ile Arg Ser Pro Asp Val Ala Asn Ile Ala Asn Ala Gln Ile
                325                 330                 335
```

```
Asp Gly His Gln Ile Ser Lys Ala Ser Tyr Glu Leu Ala Arg Gln Ser
            340                 345                 350

Phe Arg Pro Lys Leu Gln Ala Ala Tyr His Asp Tyr Phe Lys Leu His
        355                 360                 365

Gln Leu Asp Ala Ile Leu Phe Pro Thr Ala Pro Leu Thr Ala Lys Pro
    370                 375                 380

Ile Gly Gln Asp Leu Ser Val Met His Asn Gly Val Met Ala Asp Thr
385                 390                 395                 400

Phe Lys Ile Phe Val Arg Asn Val Asp Pro Gly Ser Asn Ala Gly Leu
                405                 410                 415

Pro Gly Leu Ser Leu Pro Val Ser Leu Thr Ser Lys Gly Leu Pro Ile
            420                 425                 430

Gly Met Glu Ile Asp Gly Leu Ala Gly Met Asp Asp Arg Leu Leu Ala
        435                 440                 445

Ile Gly Ala Ala Leu Glu Glu Ala Ile Ala Phe His Asn Leu Pro Asp
    450                 455                 460

Phe Pro Lys Val Glu Thr Asn Tyr
465                 470
```

<210> 37
<211> 1263
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(1260)
<223> coding for 5-methylthioribose kinase

<400> 37

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atg tct ttt gag gag ttt acg ccg tta aac gag aag tct ctt gta gac   48
Met Ser Phe Glu Glu Phe Thr Pro Leu Asn Glu Lys Ser Leu Val Asp
  1               5                  10                  15

tac atc aag tca aca cct gct ctc tct tcc aag atc gga gcc gac aag   96
Tyr Ile Lys Ser Thr Pro Ala Leu Ser Ser Lys Ile Gly Ala Asp Lys
             20                  25                  30

tcc gat gat gat ttg gtt atc aaa gaa gtt gga gat ggc aat ctc aat  144
Ser Asp Asp Asp Leu Val Ile Lys Glu Val Gly Asp Gly Asn Leu Asn
         35                  40                  45

ttc gtt ttc atc gtt gtt gga tcc tct ggt tct ctt gtc atc aaa cag  192
Phe Val Phe Ile Val Val Gly Ser Ser Gly Ser Leu Val Ile Lys Gln
     50                  55                  60

gct ctt cca tat att cgc tgt atc ggt gaa tca tgg cca atg acg aaa  240
Ala Leu Pro Tyr Ile Arg Cys Ile Gly Glu Ser Trp Pro Met Thr Lys
 65                  70                  75                  80

gaa aga gct tat ttt gaa gca aca act ttg aga aag cat gga aat tta  288
Glu Arg Ala Tyr Phe Glu Ala Thr Thr Leu Arg Lys His Gly Asn Leu
                 85                  90                  95

tca cct gat cat gtt cct gaa gtc tac cat ttt gac aga aca atg gcg  336
Ser Pro Asp His Val Pro Glu Val Tyr His Phe Asp Arg Thr Met Ala
            100                 105                 110
```

```
ttg att gga atg aga tac ctt gag cct cct cat atc att ctc cgc aaa   384
Leu Ile Gly Met Arg Tyr Leu Glu Pro Pro His Ile Ile Leu Arg Lys
        115                 120                 125

gga ctc att gct ggg att gag tat cct ttc ctc gca gac cac atg tct   432
Gly Leu Ile Ala Gly Ile Glu Tyr Pro Phe Leu Ala Asp His Met Ser
    130                 135                 140

gat tac atg gcg aag act ctc ttc ttc act tct ctc ctc tat cac gat   480
Asp Tyr Met Ala Lys Thr Leu Phe Phe Thr Ser Leu Leu Tyr His Asp
145                 150                 155                 160

acc aca gag cac aga aga gca gta acc gaa ttt tgt ggt aat gtg gag   528
Thr Thr Glu His Arg Arg Ala Val Thr Glu Phe Cys Gly Asn Val Glu
                165                 170                 175

tta tgc cga tta acg gag caa gtt gtg ttt tcg gac cca tat aga gtt   576
Leu Cys Arg Leu Thr Glu Gln Val Val Phe Ser Asp Pro Tyr Arg Val
            180                 185                 190

tcc aca ttt aat cgt tgg act tca cct tat ctt gat gat gat gct aag   624
Ser Thr Phe Asn Arg Trp Thr Ser Pro Tyr Leu Asp Asp Asp Ala Lys
        195                 200                 205

gct gtg cgc gaa gac agt gcc ttg aag ctc gaa atc gca gag cta aaa   672
Ala Val Arg Glu Asp Ser Ala Leu Lys Leu Glu Ile Ala Glu Leu Lys
    210                 215                 220

tcg atg ttc tgt gaa aga gct caa gct tta ata cat ggt gat ctt cat   720
Ser Met Phe Cys Glu Arg Ala Gln Ala Leu Ile His Gly Asp Leu His
225                 230                 235                 240

act ggt tct gtc atg gtt act caa gat tca acg caa gtt ata gat cca   768
Thr Gly Ser Val Met Val Thr Gln Asp Ser Thr Gln Val Ile Asp Pro
                245                 250                 255

gag ttt tcg ttc tat gga ccg atg ggt ttc gat att ggc gct tat ctt   816
Glu Phe Ser Phe Tyr Gly Pro Met Gly Phe Asp Ile Gly Ala Tyr Leu
            260                 265                 270

ggt aac ttg ata cta gct ttc ttt gca caa gat gga cac gcc act cag   864
Gly Asn Leu Ile Leu Ala Phe Phe Ala Gln Asp Gly His Ala Thr Gln
        275                 280                 285

gaa aat gat cga aaa gaa tac aag cag tgg atc ttg aga acc att gag   912
Glu Asn Asp Arg Lys Glu Tyr Lys Gln Trp Ile Leu Arg Thr Ile Glu
    290                 295                 300

caa act tgg aat ttg ttt aac aaa agg ttc att gcg cta tgg gat caa   960
Gln Thr Trp Asn Leu Phe Asn Lys Arg Phe Ile Ala Leu Trp Asp Gln
305                 310                 315                 320

aac aaa gat gga cca ggc gaa gca tac ctt gca gat atc tat aac aat  1008
Asn Lys Asp Gly Pro Gly Glu Ala Tyr Leu Ala Asp Ile Tyr Asn Asn
                325                 330                 335

acc gag gtt ttg aag ttt gtt caa gaa aac tac atg agg aat ttg ttg  1056
Thr Glu Val Leu Lys Phe Val Gln Glu Asn Tyr Met Arg Asn Leu Leu
            340                 345                 350

cat gac tca ctc gga ttc ggc gct gca aag atg att agg aga att gtg  1104
His Asp Ser Leu Gly Phe Gly Ala Ala Lys Met Ile Arg Arg Ile Val
        355                 360                 365

gga gtg gca cat gtt gag gac ttt gaa tca atc gaa gaa gat aag cga  1152
Gly Val Ala His Val Glu Asp Phe Glu Ser Ile Glu Glu Asp Lys Arg
    370                 375                 380
```

```
aga gct att tgc gag aga agt gca ctc gag ttt gcg aag atg ctt ctc   1200
Arg Ala Ile Cys Glu Arg Ser Ala Leu Glu Phe Ala Lys Met Leu Leu
385                 390                 395                 400

aag gaa agg aga aag ttt aag agt atc ggt gaa gtt gtt tca gca att   1248
Lys Glu Arg Arg Lys Phe Lys Ser Ile Gly Glu Val Val Ser Ala Ile
                405                 410                 415

caa caa caa agc taa                                           1263
Gln Gln Gln Ser
            420
```

<210> 38
<211> 420
<212> PRT
<213> Arabidopsis thaliana

<400> 38

```
Met Ser Phe Glu Glu Phe Thr Pro Leu Asn Glu Lys Ser Leu Val Asp
  1               5                  10                  15

Tyr Ile Lys Ser Thr Pro Ala Leu Ser Ser Lys Ile Gly Ala Asp Lys
            20                  25                  30

Ser Asp Asp Asp Leu Val Ile Lys Glu Val Gly Asp Gly Asn Leu Asn
        35                  40                  45

Phe Val Phe Ile Val Val Gly Ser Ser Gly Ser Leu Val Ile Lys Gln
    50                  55                  60

Ala Leu Pro Tyr Ile Arg Cys Ile Gly Glu Ser Trp Pro Met Thr Lys
65                  70                  75                  80

Glu Arg Ala Tyr Phe Glu Ala Thr Thr Leu Arg Lys His Gly Asn Leu
                85                  90                  95

Ser Pro Asp His Val Pro Glu Val Tyr His Phe Asp Arg Thr Met Ala
            100                 105                 110

Leu Ile Gly Met Arg Tyr Leu Glu Pro Pro His Ile Ile Leu Arg Lys
        115                 120                 125

Gly Leu Ile Ala Gly Ile Glu Tyr Pro Phe Leu Ala Asp His Met Ser
    130                 135                 140

Asp Tyr Met Ala Lys Thr Leu Phe Phe Thr Ser Leu Leu Tyr His Asp
145                 150                 155                 160

Thr Thr Glu His Arg Arg Ala Val Thr Glu Phe Cys Gly Asn Val Glu
                165                 170                 175

Leu Cys Arg Leu Thr Glu Gln Val Val Phe Ser Asp Pro Tyr Arg Val
            180                 185                 190

Ser Thr Phe Asn Arg Trp Thr Ser Pro Tyr Leu Asp Asp Asp Ala Lys
        195                 200                 205

Ala Val Arg Glu Asp Ser Ala Leu Lys Leu Glu Ile Ala Glu Leu Lys
    210                 215                 220

Ser Met Phe Cys Glu Arg Ala Gln Ala Leu Ile His Gly Asp Leu His
225                 230                 235                 240

Thr Gly Ser Val Met Val Thr Gln Asp Ser Thr Gln Val Ile Asp Pro
                245                 250                 255
```

```
Glu Phe Ser Phe Tyr Gly Pro Met Gly Phe Asp Ile Gly Ala Tyr Leu
            260                 265                 270

Gly Asn Leu Ile Leu Ala Phe Phe Ala Gln Asp Gly His Ala Thr Gln
        275                 280                 285

Glu Asn Asp Arg Lys Glu Tyr Lys Gln Trp Ile Leu Arg Thr Ile Glu
    290                 295                 300

Gln Thr Trp Asn Leu Phe Asn Lys Arg Phe Ile Ala Leu Trp Asp Gln
305                 310                 315                 320

Asn Lys Asp Gly Pro Gly Glu Ala Tyr Leu Ala Asp Ile Tyr Asn Asn
                325                 330                 335

Thr Glu Val Leu Lys Phe Val Gln Glu Asn Tyr Met Arg Asn Leu Leu
            340                 345                 350

His Asp Ser Leu Gly Phe Gly Ala Ala Lys Met Ile Arg Arg Ile Val
        355                 360                 365

Gly Val Ala His Val Glu Asp Phe Glu Ser Ile Glu Glu Asp Lys Arg
    370                 375                 380

Arg Ala Ile Cys Glu Arg Ser Ala Leu Glu Phe Ala Lys Met Leu Leu
385                 390                 395                 400

Lys Glu Arg Arg Lys Phe Lys Ser Ile Gly Glu Val Val Ser Ala Ile
                405                 410                 415

Gln Gln Gln Ser
            420
```

<210> 39
<211> 1200
<212> DNA
<213> Klebsiella pneumoniae

<220>
<221> CDS
<222> (1)..(1197)
<223> coding for 5-methylthioribose kinase

<400> 39

```
atg tcg caa tac cat acc ttc acc gcc cac gat gcc gtg gct tac gcg   48
Met Ser Gln Tyr His Thr Phe Thr Ala His Asp Ala Val Ala Tyr Ala
  1               5                  10                  15

caa cag ttc gcc ggc atc gac aac cca tct gag ctg gtc agc gcg cag   96
Gln Gln Phe Ala Gly Ile Asp Asn Pro Ser Glu Leu Val Ser Ala Gln
             20                  25                  30

gaa gtg ggc gat ggc aac ctc aat ctg gtg ttt aaa gtg ttc gat cgt   144
Glu Val Gly Asp Gly Asn Leu Asn Leu Val Phe Lys Val Phe Asp Arg
         35                  40                  45

cag ggc gtc agc cgg gcg atc gtc aaa cag gcc ctg ccc tac gtg cgc   192
Gln Gly Val Ser Arg Ala Ile Val Lys Gln Ala Leu Pro Tyr Val Arg
     50                  55                  60

tgc gtc ggc gaa tcc tgg ccg ctg acc ctc gac cgc gcc cgt ctc gaa   240
Cys Val Gly Glu Ser Trp Pro Leu Thr Leu Asp Arg Ala Arg Leu Glu
 65                  70                  75                  80
```

```
gcg cag acc ctg gtc gcc cac tat cag cac agc ccg cag cac acg gta   288
Ala Gln Thr Leu Val Ala His Tyr Gln His Ser Pro Gln His Thr Val
                85                  90                  95

aaa atc cat cac ttt gat ccc gag ctg gcg gtg atg gtg atg gaa gat   336
Lys Ile His His Phe Asp Pro Glu Leu Ala Val Met Val Met Glu Asp
            100                 105                 110

ctt tcc gac cac cgc atc tgg cgc gga gag ctt atc gct aac gtc tac   384
Leu Ser Asp His Arg Ile Trp Arg Gly Glu Leu Ile Ala Asn Val Tyr
        115                 120                 125

tat ccc cag gcg gcc cgc cag ctt ggc gac tat ctg gcg cag gtg ttg   432
Tyr Pro Gln Ala Ala Arg Gln Leu Gly Asp Tyr Leu Ala Gln Val Leu
    130                 135                 140

ttc cac acc agc gat ttc tac ctc cat ccc cac gag aaa aag gcg cag   480
Phe His Thr Ser Asp Phe Tyr Leu His Pro His Glu Lys Lys Ala Gln
145                 150                 155                 160

gtg gcg cag ttt att aac ccg gcg atg tgc gag atc acc gag gat ctg   528
Val Ala Gln Phe Ile Asn Pro Ala Met Cys Glu Ile Thr Glu Asp Leu
                165                 170                 175

ttc ttt aac gac ccg tat cag atc cac gag cgc aat aac tac ccg gcg   576
Phe Phe Asn Asp Pro Tyr Gln Ile His Glu Arg Asn Asn Tyr Pro Ala
            180                 185                 190

gag ctg gag gcc gat gtc gcc gcc ctg cgc gac gac gcc cag ctt aag   624
Glu Leu Glu Ala Asp Val Ala Ala Leu Arg Asp Asp Ala Gln Leu Lys
        195                 200                 205

ctg gcg gtg gcg gcg ctg aag cac cgt ttc ttt gcc cat gcg gaa gcg   672
Leu Ala Val Ala Ala Leu Lys His Arg Phe Phe Ala His Ala Glu Ala
    210                 215                 220

ctg ctg cac ggc gat atc cac agc ggg tcg atc ttc gtt gcc gaa ggt   720
Leu Leu His Gly Asp Ile His Ser Gly Ser Ile Phe Val Ala Glu Gly
225                 230                 235                 240

agc ctg aag gcc atc gac gcc gag ttc ggc tac ttc ggc ccc atc ggc   768
Ser Leu Lys Ala Ile Asp Ala Glu Phe Gly Tyr Phe Gly Pro Ile Gly
                245                 250                 255

ttc gat atc ggc acc gcc atc ggc aac ctg ctg ctg aac tac tgc ggc   816
Phe Asp Ile Gly Thr Ala Ile Gly Asn Leu Leu Leu Asn Tyr Cys Gly
            260                 265                 270

ctg ccg ggc cag ctc ggc att cgc gat gcc gcc gcc gcg cgc gag cag   864
Leu Pro Gly Gln Leu Gly Ile Arg Asp Ala Ala Ala Ala Arg Glu Gln
        275                 280                 285

cgg ctg aac gac atc cac cag ctg tgg acc acc ttc gcc gag cgc ttc   912
Arg Leu Asn Asp Ile His Gln Leu Trp Thr Thr Phe Ala Glu Arg Phe
    290                 295                 300

cag gcg ctg gcg gcg gag aaa acc cgc gac gcg gcg ctg gct tac ccc   960
Gln Ala Leu Ala Ala Glu Lys Thr Arg Asp Ala Ala Leu Ala Tyr Pro
305                 310                 315                 320

ggc tac gcc tcc gcc ttt ctg aag aaa gtc tgg gcg gac gcg gtc ggc  1008
Gly Tyr Ala Ser Ala Phe Leu Lys Lys Val Trp Ala Asp Ala Val Gly
                325                 330                 335

ttc tgc ggc agc gaa ctg atc cgc cgc agc gtc gga ctg tcg cac gtc  1056
Phe Cys Gly Ser Glu Leu Ile Arg Arg Ser Val Gly Leu Ser His Val
            340                 345                 350
```

```
gcg gat atc gac act atc cag gac gac gcc atg cgt cat gag tgc ctg   1104
Ala Asp Ile Asp Thr Ile Gln Asp Asp Ala Met Arg His Glu Cys Leu
        355                 360                 365

cgc cac gcc att acc ctg ggc aga gcg ctg atc gtg ctg gcc gag cgt   1152
Arg His Ala Ile Thr Leu Gly Arg Ala Leu Ile Val Leu Ala Glu Arg
    370                 375                 380

atc gac agc gtc gac gag ctg ctg gcg cgg gta cgc cag tac agc tga   1200
Ile Asp Ser Val Asp Glu Leu Leu Ala Arg Val Arg Gln Tyr Ser
385                 390                 395
```

<210> 40
<211> 399
<212> PRT
<213> Klebsiella pneumoniae

<400> 40

```
Met Ser Gln Tyr His Thr Phe Thr Ala His Asp Ala Val Ala Tyr Ala
  1               5                  10                  15

Gln Gln Phe Ala Gly Ile Asp Asn Pro Ser Glu Leu Val Ser Ala Gln
            20                  25                  30

Glu Val Gly Asp Gly Asn Leu Asn Leu Val Phe Lys Val Phe Asp Arg
        35                  40                  45

Gln Gly Val Ser Arg Ala Ile Val Lys Gln Ala Leu Pro Tyr Val Arg
    50                  55                  60

Cys Val Gly Glu Ser Trp Pro Leu Thr Leu Asp Arg Ala Arg Leu Glu
65                  70                  75                  80

Ala Gln Thr Leu Val Ala His Tyr Gln His Ser Pro Gln His Thr Val
                85                  90                  95

Lys Ile His His Phe Asp Pro Glu Leu Ala Val Met Val Met Glu Asp
            100                 105                 110

Leu Ser Asp His Arg Ile Trp Arg Gly Glu Leu Ile Ala Asn Val Tyr
        115                 120                 125

Tyr Pro Gln Ala Ala Arg Gln Leu Gly Asp Tyr Leu Ala Gln Val Leu
    130                 135                 140

Phe His Thr Ser Asp Phe Tyr Leu His Pro His Glu Lys Lys Ala Gln
145                 150                 155                 160

Val Ala Gln Phe Ile Asn Pro Ala Met Cys Glu Ile Thr Glu Asp Leu
                165                 170                 175

Phe Phe Asn Asp Pro Tyr Gln Ile His Glu Arg Asn Asn Tyr Pro Ala
            180                 185                 190

Glu Leu Glu Ala Asp Val Ala Ala Leu Arg Asp Asp Ala Gln Leu Lys
        195                 200                 205

Leu Ala Val Ala Ala Leu Lys His Arg Phe Phe Ala His Ala Glu Ala
    210                 215                 220

Leu Leu His Gly Asp Ile His Ser Gly Ser Ile Phe Val Ala Glu Gly
225                 230                 235                 240

Ser Leu Lys Ala Ile Asp Ala Glu Phe Gly Tyr Phe Gly Pro Ile Gly
                245                 250                 255

Phe Asp Ile Gly Thr Ala Ile Gly Asn Leu Leu Leu Asn Tyr Cys Gly
            260                 265                 270
```

```
Leu Pro Gly Gln Leu Gly Ile Arg Asp Ala Ala Ala Ala Arg Glu Gln
        275                 280                 285
Arg Leu Asn Asp Ile His Gln Leu Trp Thr Thr Phe Ala Glu Arg Phe
    290                 295                 300
Gln Ala Leu Ala Ala Glu Lys Thr Arg Asp Ala Ala Leu Ala Tyr Pro
305                 310                 315                 320
Gly Tyr Ala Ser Ala Phe Leu Lys Lys Val Trp Ala Asp Ala Val Gly
                325                 330                 335
Phe Cys Gly Ser Glu Leu Ile Arg Arg Ser Val Gly Leu Ser His Val
            340                 345                 350
Ala Asp Ile Asp Thr Ile Gln Asp Asp Ala Met Arg His Glu Cys Leu
        355                 360                 365
Arg His Ala Ile Thr Leu Gly Arg Ala Leu Ile Val Leu Ala Glu Arg
    370                 375                 380
Ile Asp Ser Val Asp Glu Leu Leu Ala Arg Val Arg Gln Tyr Ser
385                 390                 395
```

<210> 41
<211> 1140
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(1137)
<223> coding for alcohol dehydrogenase

<400> 41

```
atg tct acc acc gga cag att att cga tgc aaa gct gct gtg gca tgg   48
Met Ser Thr Thr Gly Gln Ile Ile Arg Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

gaa gcc gga aag cca ctg gtg atc gag gaa gtg gag gtt gct cca ccg   96
Glu Ala Gly Lys Pro Leu Val Ile Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

cag aaa cac gaa gtt cgt atc aag att ctc ttc act tct ctc tgt cac   144
Gln Lys His Glu Val Arg Ile Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

acc gat gtt tac ttc tgg gaa gct aag gga caa aca ccg ttg ttt cca   192
Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Leu Phe Pro
    50                  55                  60

cgt atc ttc ggc cat gaa gct gga ggg att gtt gag agt gtt gga gaa   240
Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
65                  70                  75                  80

gga gtg act gat ctt cag cca gga gat cat gtg ttg ccg atc ttt acc   288
Gly Val Thr Asp Leu Gln Pro Gly Asp His Val Leu Pro Ile Phe Thr
                85                  90                  95

gga gaa tgt gga gat tgt cgt cat tgc cag tcg gag gaa tca aac atg   336
Gly Glu Cys Gly Asp Cys Arg His Cys Gln Ser Glu Glu Ser Asn Met
            100                 105                 110

tgt gat ctt ctc agg atc aac aca gag cga gga ggt atg att cac gat   384
Cys Asp Leu Leu Arg Ile Asn Thr Glu Arg Gly Gly Met Ile His Asp
        115                 120                 125
```

```
ggt gaa tct aga ttc tcc att aat ggc aaa cca atc tac cat ttc ctt   432
Gly Glu Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Leu
    130                 135                 140

ggg acg tcc acg ttc agt gag tac act gtg gtt cac tct ggt cag gtc   480
Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Val His Ser Gly Gln Val
145                 150                 155                 160

gct aag atc aat ccg gat gct cct ctt gac aag gtc tgt att gtc agt   528
Ala Lys Ile Asn Pro Asp Ala Pro Leu Asp Lys Val Cys Ile Val Ser
                165                 170                 175

tgt ggt ttg tct act ggg tta gga gca act ttg aat gtg gct aaa ccc   576
Cys Gly Leu Ser Thr Gly Leu Gly Ala Thr Leu Asn Val Ala Lys Pro
            180                 185                 190

aag aaa ggt caa agt gtt gcc att ttt ggt ctt ggt gct gtt ggt tta   624
Lys Lys Gly Gln Ser Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

ggc gct gca gaa ggt gct aga atc gct ggt gct tct agg atc atc ggt   672
Gly Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

gtt gat ttt aac tct aaa aga ttc gac caa gct aag gaa ttc ggt gtg   720
Val Asp Phe Asn Ser Lys Arg Phe Asp Gln Ala Lys Glu Phe Gly Val
225                 230                 235                 240

acc gag tgt gtg aac ccg aaa gac cat gac aag cca att caa cag gtg   768
Thr Glu Cys Val Asn Pro Lys Asp His Asp Lys Pro Ile Gln Gln Val
                245                 250                 255

atc gct gag atg acg gat ggt ggg gtg gac agg agt gtg gaa tgc acc   816
Ile Ala Glu Met Thr Asp Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

gga agc gtt cag gcc atg att caa gca ttt gaa tgt gtc cac gat ggc   864
Gly Ser Val Gln Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

tgg ggt gtt gca gtg ctg gtg ggt gtg cca agc aaa gac gat gcc ttc   912
Trp Gly Val Ala Val Leu Val Gly Val Pro Ser Lys Asp Asp Ala Phe
    290                 295                 300

aag act cat ccg atg aat ttc ttg aat gag agg act ctt aag ggt act   960
Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

ttc ttc ggg aac tac aaa ccc aaa act gac att ccc ggg gtt gtg gaa  1008
Phe Phe Gly Asn Tyr Lys Pro Lys Thr Asp Ile Pro Gly Val Val Glu
                325                 330                 335

aag tac atg aac aag gag ctg gag ctt gag aaa ttc atc act cac aca  1056
Lys Tyr Met Asn Lys Glu Leu Glu Leu Glu Lys Phe Ile Thr His Thr
            340                 345                 350

gtg cca ttc tcg gaa atc aac aag gcc ttt gat tac atg ctg aag gga  1104
Val Pro Phe Ser Glu Ile Asn Lys Ala Phe Asp Tyr Met Leu Lys Gly
        355                 360                 365

gag agt att cgt tgc atc atc acc atg ggt gct tga                  1140
Glu Ser Ile Arg Cys Ile Ile Thr Met Gly Ala
    370                 375
```

<210> 42
<211> 379

<212> PRT
<213> Arabidopsis thaliana

<400> 42

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Met Ser Thr Thr Gly Gln Ile Ile Arg Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

Glu Ala Gly Lys Pro Leu Val Ile Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

Gln Lys His Glu Val Arg Ile Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Leu Phe Pro
    50                  55                  60

Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
65                  70                  75                  80

Gly Val Thr Asp Leu Gln Pro Gly Asp His Val Leu Pro Ile Phe Thr
                85                  90                  95

Gly Glu Cys Gly Asp Cys Arg His Cys Gln Ser Glu Glu Ser Asn Met
            100                 105                 110

Cys Asp Leu Leu Arg Ile Asn Thr Glu Arg Gly Gly Met Ile His Asp
        115                 120                 125

Gly Glu Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Leu
    130                 135                 140

Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Val His Ser Gly Gln Val
145                 150                 155                 160

Ala Lys Ile Asn Pro Asp Ala Pro Leu Asp Lys Val Cys Ile Val Ser
                165                 170                 175

Cys Gly Leu Ser Thr Gly Leu Gly Ala Thr Leu Asn Val Ala Lys Pro
            180                 185                 190

Lys Lys Gly Gln Ser Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

Gly Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

Val Asp Phe Asn Ser Lys Arg Phe Asp Gln Ala Lys Glu Phe Gly Val
225                 230                 235                 240

Thr Glu Cys Val Asn Pro Lys Asp His Asp Lys Pro Ile Gln Gln Val
                245                 250                 255

Ile Ala Glu Met Thr Asp Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

Gly Ser Val Gln Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

Trp Gly Val Ala Val Leu Val Gly Val Pro Ser Lys Asp Asp Ala Phe
    290                 295                 300

Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

Phe Phe Gly Asn Tyr Lys Pro Lys Thr Asp Ile Pro Gly Val Val Glu
                325                 330                 335

Lys Tyr Met Asn Lys Glu Leu Glu Leu Glu Lys Phe Ile Thr His Thr
            340                 345                 350
```

Val Pro Phe Ser Glu Ile Asn Lys Ala Phe Asp Tyr Met Leu Lys Gly
355 360 365

Glu Ser Ile Arg Cys Ile Ile Thr Met Gly Ala
370 375

<210> 43
<211> 1140
<212> DNA
<213> Hordeum vulgare

<220>
<221> CDS
<222> (1)..(1137)
<223> coding for alcohol dehydrogenase

<400> 43

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atg gcg acg gcc ggc aag gtg atc aag tgc aaa gcc gcg gtg gcg tgg   48
Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

gag gcc ggg aag ccg ctg acc atg gag gag gtg gag gtg gcg ccg ccg   96
Glu Ala Gly Lys Pro Leu Thr Met Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

cag gcc atg gag gtg cgc gtc aag atc ctc ttc acc tcc ctc tgc cac  144
Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

acc gac gtc tac ttc tgg gag gcc aag ggg cag acc ccc atg ttc cct  192
Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Met Phe Pro
    50                  55                  60

cgg atc ttc ggc cat gaa gct gga ggc ata gtg gag agt gtt gga gag  240
Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
65                  70                  75                  80

ggc gtg act gat gtt gcc cct ggt gac cac gtc ctc cct gtg ttc act  288
Gly Val Thr Asp Val Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                85                  90                  95

ggg gag tgt aag gaa tgc cca cat tgc aag tct gcg gag agc aac atg  336
Gly Glu Cys Lys Glu Cys Pro His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

tgt gat ctg ctc agg atc aac acc gac aga ggt gtg atg atc ggg gat  384
Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

ggc aag tcg cgc ttc tct att ggc ggc aag ccg att tac cat ttc gta  432
Gly Lys Ser Arg Phe Ser Ile Gly Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

ggg act tcc acc ttc agt gag tac act gtc atg cat gtc ggt tgt gtt  480
Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160

gcc aag atc aac cct gag gct ccc ctt gat aaa gtc tgt gtt ctt agc  528
Ala Lys Ile Asn Pro Glu Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

tgt ggt att tgc act ggt ctt ggc gcg tca att aat gtt gca aaa cca  576
Cys Gly Ile Cys Thr Gly Leu Gly Ala Ser Ile Asn Val Ala Lys Pro
            180                 185                 190
```

```
cca aag ggt tcc aca gtg gcg ata ttt ggg cta gga gct gtt ggc ctt   624
Pro Lys Gly Ser Thr Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

gct gct gca gaa ggt gca agg att gca ggt gca tca agg atc att ggt   672
Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

gtt gac ctg aac gcc agc aga ttt gaa gag gct agg aag ttt ggc tgc   720
Val Asp Leu Asn Ala Ser Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240

acg gaa ttt gtg aac ccg aaa gat cac acc aag cca gtt cag cag gtg   768
Thr Glu Phe Val Asn Pro Lys Asp His Thr Lys Pro Val Gln Gln Val
                245                 250                 255

ctc gct gac atg aca aat ggc gga gtt gac cgc agt gtt gag tgc act   816
Leu Ala Asp Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

ggc aac gtc aat gct atg ata caa gca ttt gaa tgt gtt cat gat ggc   864
Gly Asn Val Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

tgg ggt gta gct gtg ctg gtg ggt gtg cca cac aag gac gct gaa ttc   912
Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
    290                 295                 300

aag acc cac ccg atg aac ttc ctg aat gag agg acc ctg aag ggc acc   960
Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

ttc ttc ggt aac ttc aag ccg cgc act gac ctg ccc aat gtc gtg gag   1008
Phe Phe Gly Asn Phe Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
                325                 330                 335

atg tac atg aag aag gag ctg gag gtg gag aag ttc atc aca cac agc   1056
Met Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
            340                 345                 350

gtg ccg ttc tcg gag ata aac aag gcc ttc gac ctt atg gcg aag ggg   1104
Val Pro Phe Ser Glu Ile Asn Lys Ala Phe Asp Leu Met Ala Lys Gly
        355                 360                 365

gag ggc atc cgt tgc atc atc cgc atg gac aac tag                  1140
Glu Gly Ile Arg Cys Ile Ile Arg Met Asp Asn
    370                 375
```

<210> 44
<211> 379
<212> PRT
<213> Hordeum vulgare

<400> 44

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Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

Glu Ala Gly Lys Pro Leu Thr Met Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Met Phe Pro
    50                  55                  60
```

```
Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
 65                  70                  75                  80

Gly Val Thr Asp Val Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                85                  90                  95

Gly Glu Cys Lys Glu Cys Pro His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

Gly Lys Ser Arg Phe Ser Ile Gly Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160

Ala Lys Ile Asn Pro Glu Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

Cys Gly Ile Cys Thr Gly Leu Gly Ala Ser Ile Asn Val Ala Lys Pro
            180                 185                 190

Pro Lys Gly Ser Thr Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

Val Asp Leu Asn Ala Ser Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240

Thr Glu Phe Val Asn Pro Lys Asp His Thr Lys Pro Val Gln Gln Val
                245                 250                 255

Leu Ala Asp Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

Gly Asn Val Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
    290                 295                 300

Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

Phe Phe Gly Asn Phe Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
                325                 330                 335

Met Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
            340                 345                 350

Val Pro Phe Ser Glu Ile Asn Lys Ala Phe Asp Leu Met Ala Lys Gly
        355                 360                 365

Glu Gly Ile Arg Cys Ile Ile Arg Met Asp Asn
    370                 375
```

<210> 45
<211> 1140
<212> DNA
<213> Oryza sativa

<220>
<221> CDS

<222> (1)..(1137)
<223> coding for alcohol dehydrogenase

<400> 45

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atg gcg acc gca ggg aag gtg atc aag tgc aaa gcg gcg gtg gca tgg   48
Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

gag gcc gcg aag ccg ctg gtg atc gag gag gtg gag gtg gcg ccg ccg   96
Glu Ala Ala Lys Pro Leu Val Ile Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

cag gcc atg gag gtg cgc gtc aag atc ctc ttc acc tcg ctc tgc cac  144
Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

acc gac gtc tac ttc tgg gag gcc aag gga cag act ccc gtg ttc cct  192
Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Val Phe Pro
    50                  55                  60

cgg atc ttc ggc cat gaa gct gga ggt att gtg gag agt gtt gga gag  240
Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
65                  70                  75                  80

ggt gtg act gat ctt gcc cct ggt gac cat gtt ctc cct gtg ttc act  288
Gly Val Thr Asp Leu Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                85                  90                  95

ggg gag tgc aag gag tgt gcc cac tgc aag tca gca gag agc aac atg  336
Gly Glu Cys Lys Glu Cys Ala His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

tgt gat ctg ctc agg atc aac act gac agg ggt gtg atg att ggt gat  384
Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

ggc aaa tca cgc ttt tcc atc aac ggg aag ccc att tac cat ttc gtc  432
Gly Lys Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

ggg act tcg acc ttc agc gag tac act gtc atg cat gtt ggt tgc gtt  480
Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160

gcg aag atc aac ccg gca gct cca ctt gat aaa gtt tgc gtt ctt agc  528
Ala Lys Ile Asn Pro Ala Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

tgt ggt att tct act ggt ctt ggt gct aca atc aat gtg gca aag cca  576
Cys Gly Ile Ser Thr Gly Leu Gly Ala Thr Ile Asn Val Ala Lys Pro
            180                 185                 190

cca aag ggt tcg acg gtg gcg ata ttt ggt cta gga gct gta ggc ctt  624
Pro Lys Gly Ser Thr Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

gct gcc gca gaa ggt gca agg att gca gga gcg tca agg atc att ggc  672
Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

att gac ctg aac gcc aac aga ttt gaa gaa gct agg aaa ttt ggt tgc  720
Ile Asp Leu Asn Ala Asn Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240

act gaa ttt gtg aac cca aag gac cat gac aag cca gtt cag cag gta  768
Thr Glu Phe Val Asn Pro Lys Asp His Asp Lys Pro Val Gln Gln Val
                245                 250                 255
```

PF 53790


```
ctt gct gag atg acc aat ggc gga gtt gac cgc agc gtt gaa tgc act   816
Leu Ala Glu Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

ggc aac atc aac gcc atg atc caa gca ttt gaa tgt gtt cat gat ggc   864
Gly Asn Ile Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

tgg ggt gtt gct gtt ttg gtc ggc gtg cca cac aag gac gcc gag ttc   912
Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
    290                 295                 300

aag acc cac ccg atg aac ttc ctg aac gag agg act ctc aag gga acc   960
Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

ttc ttc ggc aac tac aag cca cgc acc gat ctg ccc aac gtc gtc gag  1008
Phe Phe Gly Asn Tyr Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
                325                 330                 335

ctc tac atg aag aag gag ctg gag gtg gag aag ttc atc aca cac agc  1056
Leu Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
            340                 345                 350

gtg ccg ttc tcg gag atc aac acg gcg ttc gac ctg atg cac aag ggc  1104
Val Pro Phe Ser Glu Ile Asn Thr Ala Phe Asp Leu Met His Lys Gly
        355                 360                 365

gag ggc atc cgc tgc atc atc cgc atg gag aac tga                 1140
Glu Gly Ile Arg Cys Ile Ile Arg Met Glu Asn
    370                 375
```

<210> 46
<211> 379
<212> PRT
<213> Oryza sativa

<400> 46

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Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

Glu Ala Ala Lys Pro Leu Val Ile Glu Glu Val Glu Val Ala Pro Pro
             20                  25                  30

Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
         35                  40                  45

Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Val Phe Pro
     50                  55                  60

Arg Ile Phe Gly His Glu Ala Gly Gly Ile Val Glu Ser Val Gly Glu
 65                  70                  75                  80

Gly Val Thr Asp Leu Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                 85                  90                  95

Gly Glu Cys Lys Glu Cys Ala His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

Gly Lys Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160
```

```
Ala Lys Ile Asn Pro Ala Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

Cys Gly Ile Ser Thr Gly Leu Gly Ala Thr Ile Asn Val Ala Lys Pro
            180                 185                 190

Pro Lys Gly Ser Thr Val Ala Ile Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

Ile Asp Leu Asn Ala Asn Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240

Thr Glu Phe Val Asn Pro Lys Asp His Asp Lys Pro Val Gln Gln Val
                245                 250                 255

Leu Ala Glu Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

Gly Asn Ile Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
    290                 295                 300

Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320

Phe Phe Gly Asn Tyr Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
                325                 330                 335

Leu Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
            340                 345                 350

Val Pro Phe Ser Glu Ile Asn Thr Ala Phe Asp Leu Met His Lys Gly
        355                 360                 365

Glu Gly Ile Arg Cys Ile Ile Arg Met Glu Asn
    370                 375
```

<210> 47
<211> 1140
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)..(1137)
<223> coding for alcohol dehydrogenase

<400> 47

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atg gcg acc gcg ggg aag gtg atc aag tgc aaa gct gcg gtg gca tgg   48
Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

gag gcc ggc aag cca ctg tcg atc gag gag gtg gag gta gcg cct ccg   96
Glu Ala Gly Lys Pro Leu Ser Ile Glu Glu Val Glu Val Ala Pro Pro
             20                  25                  30

cag gcc atg gag gtg cgc gtc aag atc ctc ttc acc tcg ctc tgc cac  144
Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
         35                  40                  45
```

```
acc gac gtc tac ttc tgg gag gcc aag ggg cag act ccc gtg ttc cct   192
Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Val Phe Pro
     50                  55                  60

cgg atc ttt ggc cat gag gct gga ggt atc ata gag agt gtt gga gag   240
Arg Ile Phe Gly His Glu Ala Gly Gly Ile Ile Glu Ser Val Gly Glu
 65                  70                  75                  80

ggt gtg act gac gta gct ccg ggc gac cat gtc ctt cct gtg ttc act   288
Gly Val Thr Asp Val Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                 85                  90                  95

ggg gag tgc aag gag tgc gcc cac tgc aag tcg gca gag agc aac atg   336
Gly Glu Cys Lys Glu Cys Ala His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

tgt gat ttg ctc agg atc aac act gac cgc ggt gtg atg att ggc gat   384
Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

ggc aag tcg cgg ttt tca atc aat ggg aag cct atc tac cac ttt gtt   432
Gly Lys Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

ggg act tcc acc ttc agc gag tac acc gtc atg cat gtc ggt tgt gtt   480
Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160

gca aag atc aac cct cag gct ccc ctt gat aaa gtt tgc gtc ctt agc   528
Ala Lys Ile Asn Pro Gln Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

tgt ggt att tct act ggt ctt ggt gca tca att aat gtt gca aaa cct   576
Cys Gly Ile Ser Thr Gly Leu Gly Ala Ser Ile Asn Val Ala Lys Pro
            180                 185                 190

ccg aag ggt tcg aca gtg gct gtt ttc ggt tta gga gcc gtt ggt ctt   624
Pro Lys Gly Ser Thr Val Ala Val Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

gcc gct gca gaa ggt gca agg att gct gga gcg tca agg atc att ggt   672
Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

gtc gac ctg aac ccc agc aga ttc gaa gaa gct agg aag ttc ggt tgc   720
Val Asp Leu Asn Pro Ser Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240

act gaa ttt gtg aac cca aaa gac cac aac aag ccg gtg cag gag gta   768
Thr Glu Phe Val Asn Pro Lys Asp His Asn Lys Pro Val Gln Glu Val
                245                 250                 255

ctt gct gag atg acc aac gga ggg gtc gac cgc agc gtg gaa tgc act   816
Leu Ala Glu Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
            260                 265                 270

ggc aac atc aat gct atg atc caa gct ttc gaa tgt gtt cat gat ggc   864
Gly Asn Ile Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
        275                 280                 285

tgg ggt gtt gcc gtg ctg gtg ggt gtg ccg cat aag gac gct gag ttc   912
Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
    290                 295                 300

aag acc cac ccg atg aac ttc ctg aac gaa agg acc ctg aag ggg acc   960
Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305                 310                 315                 320
```

```
ttc ttt ggc aac tat aag cca cgc act gat ctg cca aat gtg gtg gag   1008
Phe Phe Gly Asn Tyr Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
                325                 330                 335

ctg tac atg aaa aag gag ctg gag gtg gag aag ttc atc acg cac agc   1056
Leu Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
            340                 345                 350

gtc ccg ttc gcg gag atc aac aag gcg ttc aac ctg atg gcc aag ggg   1104
Val Pro Phe Ala Glu Ile Asn Lys Ala Phe Asn Leu Met Ala Lys Gly
        355                 360                 365

gag ggc atc cgc tgc atc atc cgc atg gag aac tag                   1140
Glu Gly Ile Arg Cys Ile Ile Arg Met Glu Asn
    370                 375
```

<210> 48
<211> 379
<212> PRT
<213> Zea mays

<400> 48

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Met Ala Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Ala Trp
  1               5                  10                  15

Glu Ala Gly Lys Pro Leu Ser Ile Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

Gln Ala Met Glu Val Arg Val Lys Ile Leu Phe Thr Ser Leu Cys His
        35                  40                  45

Thr Asp Val Tyr Phe Trp Glu Ala Lys Gly Gln Thr Pro Val Phe Pro
    50                  55                  60

Arg Ile Phe Gly His Glu Ala Gly Gly Ile Ile Glu Ser Val Gly Glu
65                  70                  75                  80

Gly Val Thr Asp Val Ala Pro Gly Asp His Val Leu Pro Val Phe Thr
                85                  90                  95

Gly Glu Cys Lys Glu Cys Ala His Cys Lys Ser Ala Glu Ser Asn Met
            100                 105                 110

Cys Asp Leu Leu Arg Ile Asn Thr Asp Arg Gly Val Met Ile Gly Asp
        115                 120                 125

Gly Lys Ser Arg Phe Ser Ile Asn Gly Lys Pro Ile Tyr His Phe Val
    130                 135                 140

Gly Thr Ser Thr Phe Ser Glu Tyr Thr Val Met His Val Gly Cys Val
145                 150                 155                 160

Ala Lys Ile Asn Pro Gln Ala Pro Leu Asp Lys Val Cys Val Leu Ser
                165                 170                 175

Cys Gly Ile Ser Thr Gly Leu Gly Ala Ser Ile Asn Val Ala Lys Pro
            180                 185                 190

Pro Lys Gly Ser Thr Val Ala Val Phe Gly Leu Gly Ala Val Gly Leu
        195                 200                 205

Ala Ala Ala Glu Gly Ala Arg Ile Ala Gly Ala Ser Arg Ile Ile Gly
    210                 215                 220

Val Asp Leu Asn Pro Ser Arg Phe Glu Glu Ala Arg Lys Phe Gly Cys
225                 230                 235                 240
```

Thr Glu Phe Val Asn Pro Lys Asp His Asn Lys Pro Val Gln Glu Val
245 250 255

Leu Ala Glu Met Thr Asn Gly Gly Val Asp Arg Ser Val Glu Cys Thr
260 265 270

Gly Asn Ile Asn Ala Met Ile Gln Ala Phe Glu Cys Val His Asp Gly
275 280 285

Trp Gly Val Ala Val Leu Val Gly Val Pro His Lys Asp Ala Glu Phe
290 295 300

Lys Thr His Pro Met Asn Phe Leu Asn Glu Arg Thr Leu Lys Gly Thr
305 310 315 320

Phe Phe Gly Asn Tyr Lys Pro Arg Thr Asp Leu Pro Asn Val Val Glu
325 330 335

Leu Tyr Met Lys Lys Glu Leu Glu Val Glu Lys Phe Ile Thr His Ser
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Val Pro Phe Ala Glu Ile Asn Lys Ala Phe Asn Leu Met Ala Lys Gly
355 360 365

Glu Gly Ile Arg Cys Ile Ile Arg Met Glu Asn
370 375

<210> 49
<211> 505
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<213> Artificial sequence

<220>
<223> Description of the artificial sequence: coding for sense RNA-fragment of E.coli codA gene

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aatccggcgt gatgcccata actgaaaaca gcctggatgc cgaacaaggt ttagttatac 180
cgccgtttgt ggagccacat attcacctgg acaccacgca aaccgccgga caaccgaact 240
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gcattcagca tgtgcgtacc catgtcgatg tttcggatgc aacgctaact gcgctgaaag 420
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ctcaggaagg gattttgtcg tcgac 505

<210> 50
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: oligonucleotide primer

<400> 50
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<210> 51
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
oligonucleotide primer

<400> 51
cggcaggata atcaggttgg 20

<210> 52
<211> 505
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: coding for
antisense RNA-fragment of E.coli codA gene

<400> 52
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<210> 53
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
oligonucleotide primer

<400> 53
gtcaacgtaa ccaaccctgc 20

<210> 54
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
oligonucleotide primer

<400> 54
ggatccgaca aaatcccttc ctgagg 26

<210> 55
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<212> DNA
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<223> Description of the artificial sequence: vector
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<211> 6046
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: binary vector pSUN1

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<211> 9838
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: Transgenic expression vector for codA dsRNA pSUN1-codA-RNAi

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<211> 14184
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: Expression vector pSUN1-codA-RNAi-At.Act.-2-At.Als-R-ocsT

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Leu Asp Glu Ser Ser Leu Leu Ala Tyr Ile Lys Ala Thr Pro Ala Leu
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gcc tcc cgc ctc ggc ggc ggt ggc agt cta gac tcc atc gag atc aag  192
Ala Ser Arg Leu Gly Gly Gly Gly Ser Leu Asp Ser Ile Glu Ile Lys
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Glu Val Gly Asp Gly Asn Leu Asn Phe Val Tyr Ile Val Gln Ser Glu
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Gly Asp Ser Trp Pro Met Thr Arg Glu Arg Ala Tyr Phe Glu Ala Ser
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Thr Leu Arg Glu His Gly Arg Leu Cys Pro Glu His Thr Pro Glu Val
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Tyr His Phe Asp Arg Thr Leu Ser Leu Met Gly Met Arg Tyr Ile Glu
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Pro Pro His Ile Ile Leu Arg Lys Gly Leu Val Ala Gly Val Glu Tyr
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Pro Tyr Leu Asp Lys Asp Ala Glu Ala Val Arg Glu Asp Asp Glu Leu
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Lys Leu Glu Val Ala Gly Leu Lys Ser Met Phe Ile Glu Arg Ala Gln
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Ala Leu Ile His Gly Asp Leu His Thr Gly Ser Ile Met Val Thr Glu
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Val Gln Leu Lys Ser Leu Ile Gln Asn Leu Gly Ser Met Gly Pro Met
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ggg ttt gat att ggg agc ctt cct tgg aaa cct gat ttt ggg cat act   912
Gly Phe Asp Ile Gly Ser Leu Pro Trp Lys Pro Asp Phe Gly His Thr
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         35                  40                  45

Ala Ser Arg Leu Gly Gly Gly Gly Ser Leu Asp Ser Ile Glu Ile Lys
     50                  55                  60

Glu Val Gly Asp Gly Asn Leu Asn Phe Val Tyr Ile Val Gln Ser Glu
 65                  70                  75                  80

Ala Gly Ala Ile Val Val Lys Gln Ala Leu Pro Tyr Val Arg Cys Val
                 85                  90                  95

Gly Asp Ser Trp Pro Met Thr Arg Glu Arg Ala Tyr Phe Glu Ala Ser
            100                 105                 110

Thr Leu Arg Glu His Gly Arg Leu Cys Pro Glu His Thr Pro Glu Val
        115                 120                 125
```

```
Tyr His Phe Asp Arg Thr Leu Ser Leu Met Gly Met Arg Tyr Ile Glu
    130                 135                 140

Pro Pro His Ile Ile Leu Arg Lys Gly Leu Val Ala Gly Val Glu Tyr
145                 150                 155                 160

Pro Leu Leu Ala Asp His Met Ser Asp Tyr Met Ala Lys Thr Leu Phe
                165                 170                 175

Phe Thr Ser Leu Leu Tyr Asn Asn Thr Thr Asp His Lys Asn Gly Val
            180                 185                 190

Ala Lys Tyr Ser Ala Asn Val Glu Met Cys Arg Leu Thr Glu Gln Val
        195                 200                 205

Val Phe Ser Asp Pro Tyr Arg Val Ser Lys Phe Asn Arg Trp Thr Ser
    210                 215                 220

Pro Tyr Leu Asp Lys Asp Ala Glu Ala Val Arg Glu Asp Asp Glu Leu
225                 230                 235                 240

Lys Leu Glu Val Ala Gly Leu Lys Ser Met Phe Ile Glu Arg Ala Gln
                245                 250                 255

Ala Leu Ile His Gly Asp Leu His Thr Gly Ser Ile Met Val Thr Glu
            260                 265                 270

Val Gln Leu Lys Ser Leu Ile Gln Asn Leu Gly Ser Met Gly Pro Met
        275                 280                 285

Gly Phe Asp Ile Gly Ser Leu Pro Trp Lys Pro Asp Phe Gly His Thr
    290                 295                 300

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Arg Met Asp Leu Glu Asp Asn
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<220>
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<223> coding for 5-methylthioribose kinase

<400> 61

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  Phe Pro Gly Arg Arg Phe Arg Gly Asn Leu Asn Phe Val Phe Ile Val
    1               5                  10                  15

atc gga tcc act ggc tca ctc gtc atc aaa cag gcg ctt ccg tat ata   97
Ile Gly Ser Thr Gly Ser Leu Val Ile Lys Gln Ala Leu Pro Tyr Ile
            20                  25                  30

cgt tgt att ggg gag tct tgg cca atg acg aaa gaa aga gct tac ttt   145
Arg Cys Ile Gly Glu Ser Trp Pro Met Thr Lys Glu Arg Ala Tyr Phe
        35                  40                  45

gaa gct aca act ctg aga aag cac gga gct ttg tct cct gat cat gtt   193
Glu Ala Thr Thr Leu Arg Lys His Gly Ala Leu Ser Pro Asp His Val
    50                  55                  60
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cct gaa gtc tac cat ttt gac agg acc atg gct ttg att gga atg agg   241
Pro Glu Val Tyr His Phe Asp Arg Thr Met Ala Leu Ile Gly Met Arg
 65                  70                  75                  80

tat ctg gag cct cct cac atc atc ctc cgc aaa gga ctc gtt gct gga   289
Tyr Leu Glu Pro Pro His Ile Ile Leu Arg Lys Gly Leu Val Ala Gly
                85                  90                  95

atc cag tac cct ttc ctt gca gaa cac atg gct gat tac atg gcc aaa   337
Ile Gln Tyr Pro Phe Leu Ala Glu His Met Ala Asp Tyr Met Ala Lys
            100                 105                 110

acc ctc ttc ttc act tcg ctc ctc tat cat gat acc aca gag cac aaa   385
Thr Leu Phe Phe Thr Ser Leu Leu Tyr His Asp Thr Thr Glu His Lys
        115                 120                 125

aga gca gta acc gag ttt tgt ggt aat gtg gag tta tgc cgg tta acg   433
Arg Ala Val Thr Glu Phe Cys Gly Asn Val Glu Leu Cys Arg Leu Thr
    130                 135                 140

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             20                  25                  30

Arg Cys Ile Gly Glu Ser Trp Pro Met Thr Lys Glu Arg Ala Tyr Phe
         35                  40                  45

Glu Ala Thr Thr Leu Arg Lys His Gly Ala Leu Ser Pro Asp His Val
     50                  55                  60

Pro Glu Val Tyr His Phe Asp Arg Thr Met Ala Leu Ile Gly Met Arg
 65                  70                  75                  80

Tyr Leu Glu Pro Pro His Ile Ile Leu Arg Lys Gly Leu Val Ala Gly
                85                  90                  95

Ile Gln Tyr Pro Phe Leu Ala Glu His Met Ala Asp Tyr Met Ala Lys
            100                 105                 110

Thr Leu Phe Phe Thr Ser Leu Leu Tyr His Asp Thr Thr Glu His Lys
        115                 120                 125

Arg Ala Val Thr Glu Phe Cys Gly Asn Val Glu Leu Cys Arg Leu Thr
    130                 135                 140

Glu Gln Val Val Phe Ser Asp Pro Tyr Arg Val Ser
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1 5 10 15

ttc aag ccg ttg aac gag aaa tct cta gta gag tac ata aag gca acg 95
Phe Lys Pro Leu Asn Glu Lys Ser Leu Val Glu Tyr Ile Lys Ala Thr
20 25 30

cct gcc ctc tcc tcc agg ctc gga gac aag tac gat gat ctg gtc atc 143
Pro Ala Leu Ser Ser Arg Leu Gly Asp Lys Tyr Asp Asp Leu Val Ile
35 40 45

aag gaa gtt gga gat ggc aat ctc aac ttc gtt ttc atc gtt gtc gga 191
Lys Glu Val Gly Asp Gly Asn Leu Asn Phe Val Phe Ile Val Val Gly
50 55 60

tcc act ggc tca ctc gtc atc aaa cag gcg ctt ccg tat ata cgt tgt 239
Ser Thr Gly Ser Leu Val Ile Lys Gln Ala Leu Pro Tyr Ile Arg Cys
65 70 75

att gga gaa tca tgg cca atg acg aaa gaa aga gct tac ttt gaa gca 287
Ile Gly Glu Ser Trp Pro Met Thr Lys Glu Arg Ala Tyr Phe Glu Ala
80 85 90 95

aca act ctg aga aag cac ggt ggt ttg tct ccg gat cat gtt cct gaa 335
Thr Thr Leu Arg Lys His Gly Gly Leu Ser Pro Asp His Val Pro Glu
100 105 110

gtc tac cat ttt gac aga acc atg gct ttg att gga atg aga tac ctc 383
Val Tyr His Phe Asp Arg Thr Met Ala Leu Ile Gly Met Arg Tyr Leu
115 120 125

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20 25 30

Ala Leu Ser Ser Arg Leu Gly Asp Lys Tyr Asp Asp Leu Val Ile Lys
35 40 45

Glu Val Gly Asp Gly Asn Leu Asn Phe Val Phe Ile Val Val Gly Ser
50 55 60

Thr Gly Ser Leu Val Ile Lys Gln Ala Leu Pro Tyr Ile Arg Cys Ile
65 70 75 80

Gly Glu Ser Trp Pro Met Thr Lys Glu Arg Ala Tyr Phe Glu Ala Thr
85 90 95

PF 53790


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Thr Leu Arg Lys His Gly Gly Leu Ser Pro Asp His Val Pro Glu Val
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Tyr His Phe Asp Arg Thr Met Ala Leu Ile Gly Met Arg Tyr Leu Glu
        115                 120                 125

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tac tgc gat aat gtg gag atg tgt agg ctc aca gag caa gtc gtg ttc   95
Tyr Cys Asp Asn Val Glu Met Cys Arg Leu Thr Glu Gln Val Val Phe
                20                  25                  30

tca gac cca tac atg ctc gcc aaa tac aat cgt tgc aca tca ccc ttc   143
Ser Asp Pro Tyr Met Leu Ala Lys Tyr Asn Arg Cys Thr Ser Pro Phe
            35                  40                  45

cta gat aat gat gct gca gcg gtt cga gag gat gct gag ctt aaa ttg   191
Leu Asp Asn Asp Ala Ala Ala Val Arg Glu Asp Ala Glu Leu Lys Leu
        50                  55                  60

gag att gct gaa ttg aaa tca atg ttt att gag aga gca cag gct ctt   239
Glu Ile Ala Glu Leu Lys Ser Met Phe Ile Glu Arg Ala Gln Ala Leu
    65                  70                  75

ctt cat gga gat ctc cac act ggt tcc atc atg gtg aca cca gat tct   287
Leu His Gly Asp Leu His Thr Gly Ser Ile Met Val Thr Pro Asp Ser
80                  85                  90                  95

act caa gtg att gat cca gaa ttt gct ttc tat ggc cca atg ggt tac   335
Thr Gln Val Ile Asp Pro Glu Phe Ala Phe Tyr Gly Pro Met Gly Tyr
                100                 105                 110

gac att ggg gcc ttc ctg ggg aac ttg att ttg gca tat ttt tca caa   383
Asp Ile Gly Ala Phe Leu Gly Asn Leu Ile Leu Ala Tyr Phe Ser Gln
            115                 120                 125

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        130                 135                 140
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  1               5                  10                  15
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PF 53790


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        35                  40                  45
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    50                  55                  60
Ile Ala Glu Leu Lys Ser Met Phe Ile Glu Arg Ala Gln Ala Leu Leu
65                  70                  75                  80
His Gly Asp Leu His Thr Gly Ser Ile Met Val Thr Pro Asp Ser Thr
                85                  90                  95
Gln Val Ile Asp Pro Glu Phe Ala Phe Tyr Gly Pro Met Gly Tyr Asp
            100                 105                 110
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tct ttg atc ggt atg cgt tac ttg gag ccc cca cat ata atc ctc ata   95
Ser Leu Ile Gly Met Arg Tyr Leu Glu Pro Pro His Ile Ile Leu Ile
                20                  25                  30
aaa ggg ttg att gct ggg att gag tac cct ttt ttg gct gaa cac atg   143
Lys Gly Leu Ile Ala Gly Ile Glu Tyr Pro Phe Leu Ala Glu His Met
            35                  40                  45
gct gat ttc atg gcg aag aca ctc ttc ttc acg tct ctg ctt ttc cgt   191
Ala Asp Phe Met Ala Lys Thr Leu Phe Phe Thr Ser Leu Leu Phe Arg
        50                  55                  60
tcc act gct gac cac aaa cgg gac gtt gcc gaa ttt tgt ggg aat gtg   239
Ser Thr Ala Asp His Lys Arg Asp Val Ala Glu Phe Cys Gly Asn Val
    65                  70                  75
gag tta tgc agg ctc act gaa cag gtc gtt ttc tct gac cct tat aaa   287
Glu Leu Cys Arg Leu Thr Glu Gln Val Val Phe Ser Asp Pro Tyr Lys
80                  85                  90                  95
gtt tct caa tat aat cgt tgg act tcc ccc tat ctt gat cgt gat gct   335
Val Ser Gln Tyr Asn Arg Trp Thr Ser Pro Tyr Leu Asp Arg Asp Ala
                100                 105                 110
gag gct gtt cgg gaa gac aat ctg ctg aag ctt gaa gtt gct gag ctg   383
Glu Ala Val Arg Glu Asp Asn Leu Leu Lys Leu Glu Val Ala Glu Leu
            115                 120                 125
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130

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20 25 30

Gly Leu Ile Ala Gly Ile Glu Tyr Pro Phe Leu Ala Glu His Met Ala
35 40 45

Asp Phe Met Ala Lys Thr Leu Phe Phe Thr Ser Leu Leu Phe Arg Ser
50 55 60

Thr Ala Asp His Lys Arg Asp Val Ala Glu Phe Cys Gly Asn Val Glu
65 70 75 80

Leu Cys Arg Leu Thr Glu Gln Val Val Phe Ser Asp Pro Tyr Lys Val
85 90 95

Ser Gln Tyr Asn Arg Trp Thr Ser Pro Tyr Leu Asp Arg Asp Ala Glu
100 105 110

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115 120 125

Ser Lys Phe Ile Glu Ser
130

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We claim:

1. A process for preparing transformed plant cells or organisms, which comprises the following steps:

a) transforming a population of plant cells, with the cells of said population containing at least one marker protein capable of causing directly or indirectly a toxic effect for said population, with at least one nucleic acid sequence to be inserted in combination with at least one double-stranded marker protein ribonucleic acid sequence or an expression cassette or expression cassettes ensuring expression thereof capable of reducing the expression of at least one marker protein, and

b) selecting transformed plant cells whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said double-stranded marker protein ribonucleic acid sequence, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells.

2. The process as claimed in claim 1, wherein the marker protein is capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population, which process comprises the following steps:

a) transforming the population of plant cells with at least one nucleic acid sequence to be inserted in combination with at least one double-stranded marker protein ribonucleic acid sequence or an expression cassette or expression cassettes ensuring expression thereof capable of reducing the expression of at least one marker protein, and

b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and

c) selecting transformed plant cells whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said

double-stranded marker protein ribonucleic acid sequence, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells.

3. The process as claimed in claim 2, wherein the nontoxic substance X is a substance which does not naturally occur in plant cells or organisms or occurs naturally therein only at a concentration which can essentially not cause any toxic effect.

4. The process as claimed in claim 2 or 3, wherein the substance X is a substance selected from the group consisting of proherbicides, proantibiotics, nucleoside analogs, 5-fluorocytosine, auxinamide compounds, naphthalacetamide, dihaloalkanes, Acyclovir, Ganciclovir, 1,2-deoxy-2-fluoro-b-D-arabinofuranosil-5-iodouracil, 6-thioxanthine, allopurinol, 6-methylpurine deoxyribonucleoside, 4-aminopyrazolopyrimidine, 2-amino-4-methoxybutanoic acid, 5-(trifluoromethyl)thioribose and allyl alcohol.

5. The process as claimed in any of claims 1 to 4, wherein the marker protein is selected from the group consisting of cytosine deaminases, cytochrome P-450 enzymes, indoleacetic acid hydrolases, haloalkane dehalogenases, thymidine kinases, guanine phosphoribosyl transferases, hypoxanthine phosphoribosyl transferases, xanthine guanine phosphoribosyl transferases, purine nucleoside phosphorylases, phosphonate monoester hydrolases, indoleacetamide synthases, indoleacetamide hydrolases, adenine phosphoribosyl transferases, methoxinine dehydrogenases, rhizobitoxin synthases, 5-methylthioribose kinases and alcohool dehydrogenases.

6. The process as claimed in any of claims 1 to 5, wherein the marker protein is encoded by

a) a sequence described by the GenBank accession number S56903, M32238, NC003308, AE009419, AB016260, NC002147, M26950, J02224, V00470, V00467, U10247, M13422, X00221, M60917, U44852, M61151, AF039169, AB025110, AF212863, AC079674, X77943, M12196, AF172282, X04049 or AF253472

b) a sequence according to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40,

42, 44, 46 or 48.

7. The process as claimed in any of claims 1 to 6, wherein a sequence coding for a resistance to at lesat one toxin, antibiotic or herbicide is introduced together with the nucleic acid sequence to be inserted and selection is carried out additionally using the toxin, antibiotic or herbicide.

8. The process as claimed in any of claims 1 to 7, wherein the nucleic acid sequence to be inserted into the genome of the plant cell or of the plant organism comprises at least one expression cassette capable of expressing, under the control of a promoter functional in plant cells or in plant organisms, an RNA and/or a protein which does not cause the expression, amount, activity and/or function of a marker protein to be reduced.

9. The process as claimed in any of claims 1 to 8, wherein the plant cell is part of a plant organism or of a tissue, part, organ, cell culture or propagation material derived therefrom.

10. The process as claimed in any of claims 1 to 9 for preparing transformed plant cells or organisms, which comprises the following steps:

a) transforming a population of plant cells which comprises at least one non-endogenous (preferably non-plant) marker protein capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population, with at least one nucleic acid sequence to be inserted in combination with at least one nucleic acid sequence coding for a double-stranded marker protein ribonucleic acid sequence or an expression cassette or expression cassettes ensuring expression thereof ribonucleic acid sequence capable of reducing the expression, amount, activity and/or function of said marker protein, and

b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and

c) selecting transformed plant cells (and/or populations of plant cells, such as plant tissues or plants) whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said double-stranded marker protein ribonucleic acid sequence, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells, and

d) regenerating fertile plants, and

e) eliminating by crossing the nucleic acid sequence coding for the marker protein and isolating fertile plants whose genome contains said nucleic acid sequence but does not contain any longer the sequence coding for the marker protein.

11. An amino acid sequence coding for a plant 5-methylthioribose kinase, wherein said amino acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 60, 62, 64, 66 or 68.

12. A nucleic acid sequence coding for a plant 5-methylthioribose kinase, wherein said nucleic acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 59, 61, 63, 65 or 67.

13. A double-stranded RNA molecule, comprising

a) a "sense" RNA strand comprising at least one ribonucleotide sequence which is essentially identical to at least a part of the "sense" RNA transcript of a nucleic acid sequence coding for a marker protein, and

b) an "antisense" RNA strand which is essentially, preferably fully, complementary to the RNA sense strand under a).

14. The double-stranded RNA molecule as claimed in claim 13, wherein the marker protein is defined as in any of claims 2 to 6.

15. The double-stranded RNA molecule as claimed in either of claims 13 and 14, wherein the "sense" RNA strand and the "an-

tisense" RNA strand are covalently linked to one another in the form of an inverted repeat.

16. A transgenic expression cassette, comprising a nucleic acid sequence which codes for a double-stranded RNA molecule as claimed in any of claims 13 to 15 and which is functionally linked to a promoter functional in plant organisms.

17. A transgenic vector, comprising a transgenic expression cassette as claimed in claim 16.

18. A transgenic plant organism, comprising a double-stranded RNA molecule as claimed in any of claims 13 to 15, a transgenic expression cassette as claimed in claim 16 or a transgenic vector as claimed in claim 17.

19. The transgenic plant organism as claimed in claim 18, selected from the group of plants, consisting of wheat, oats, millet, barley, rye, corn, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, oilseed rape, cress, arabidopsis, cabbage species, soybean, alfalfa, pea, bean plants, peanut, potato, tobacco, tomato, eggplant, paprika, sunflower, tagetes, lettuce, calendula, melon, pumpkin and zucchini.

20. A tissue, an organ, a part, a cell, a cell culture or propagation material, derived from a transgenic plant organism as claimed in either of claims 18 and 19.

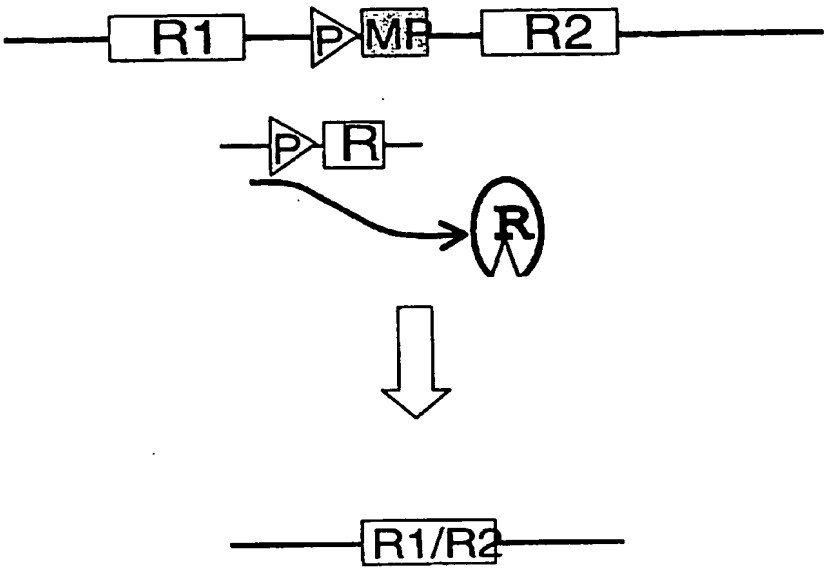

Fig. 1

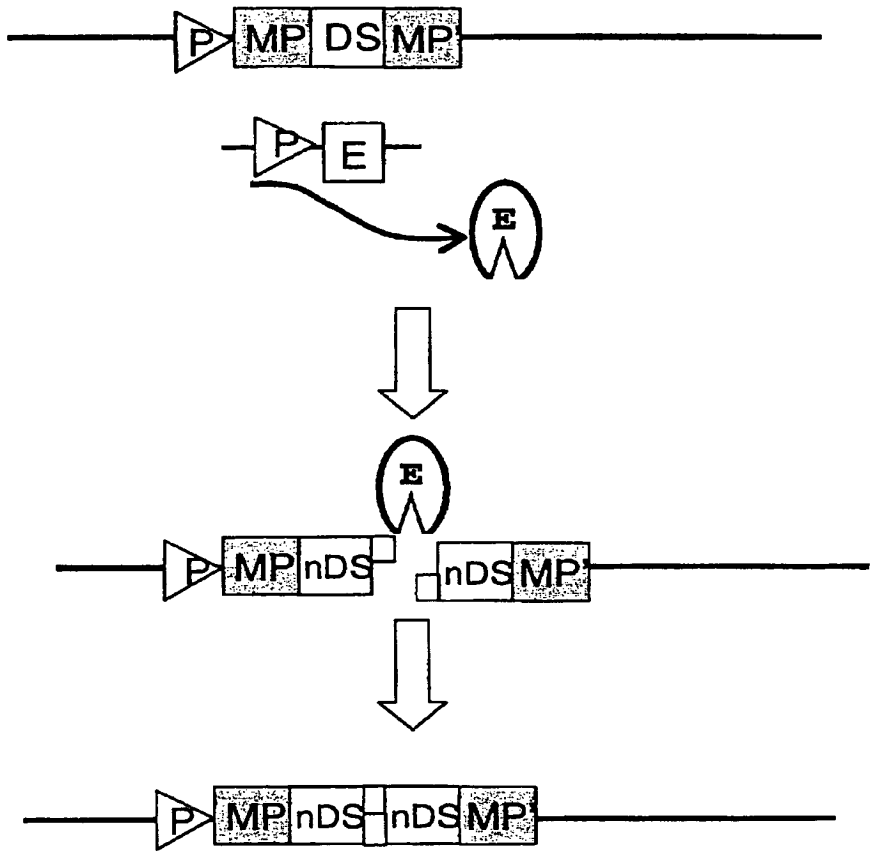

Fig. 2–A

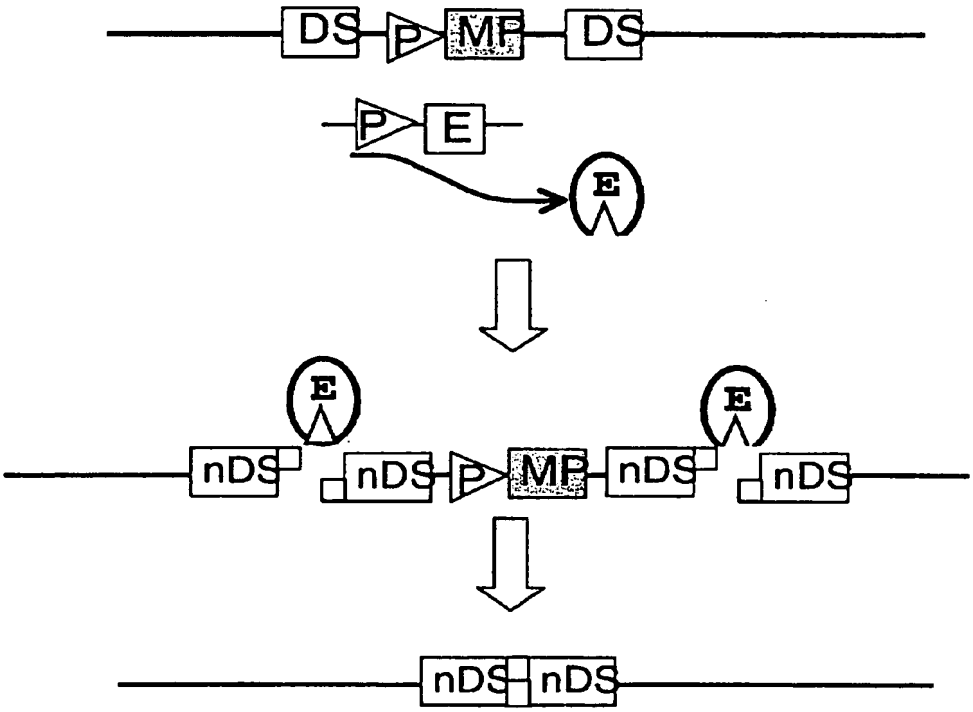

Fig. 2–B

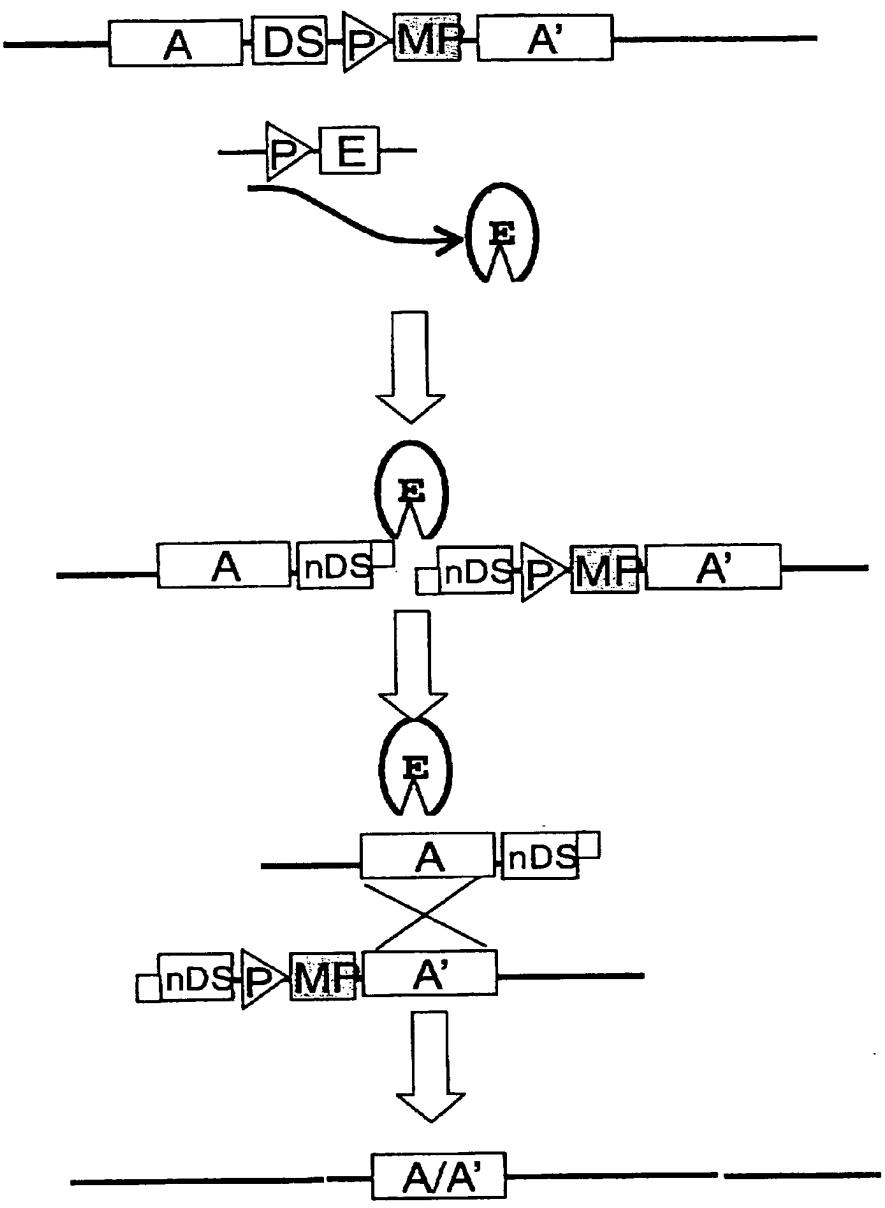

Fig. 3

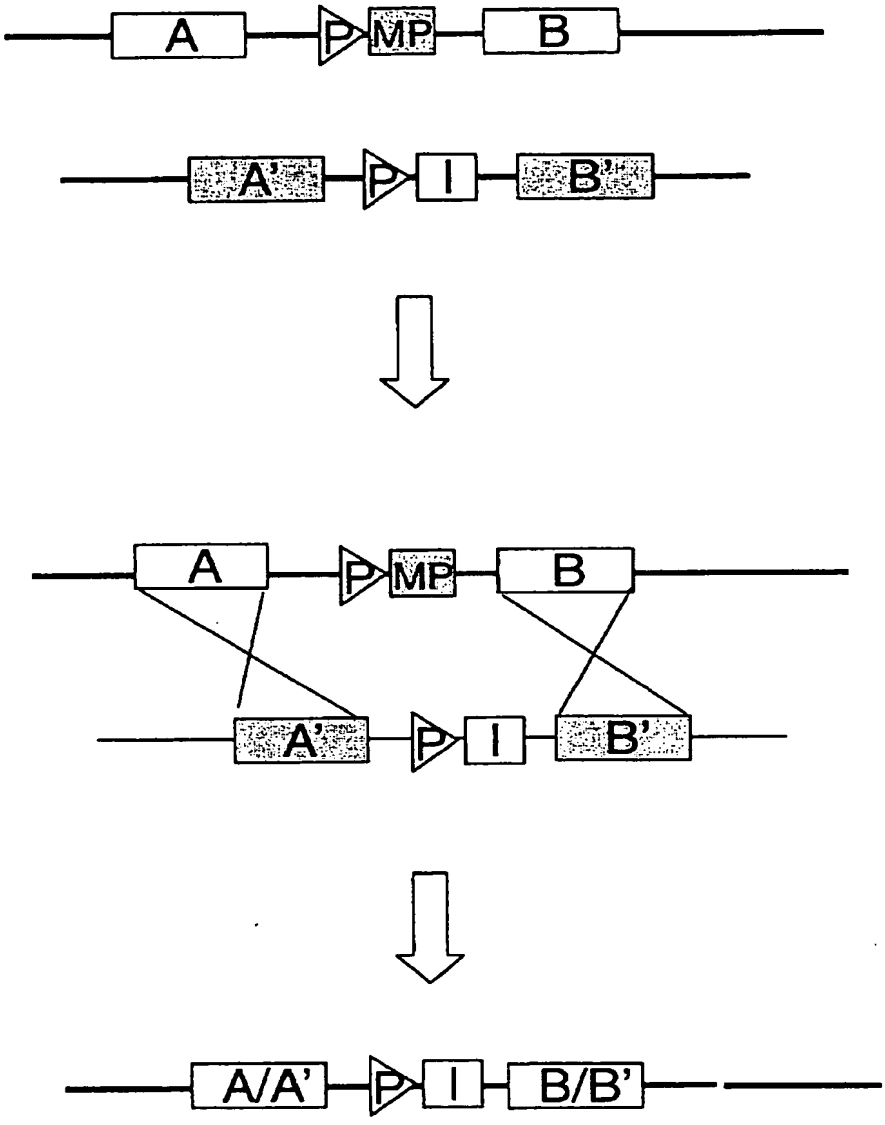

Fig. 4

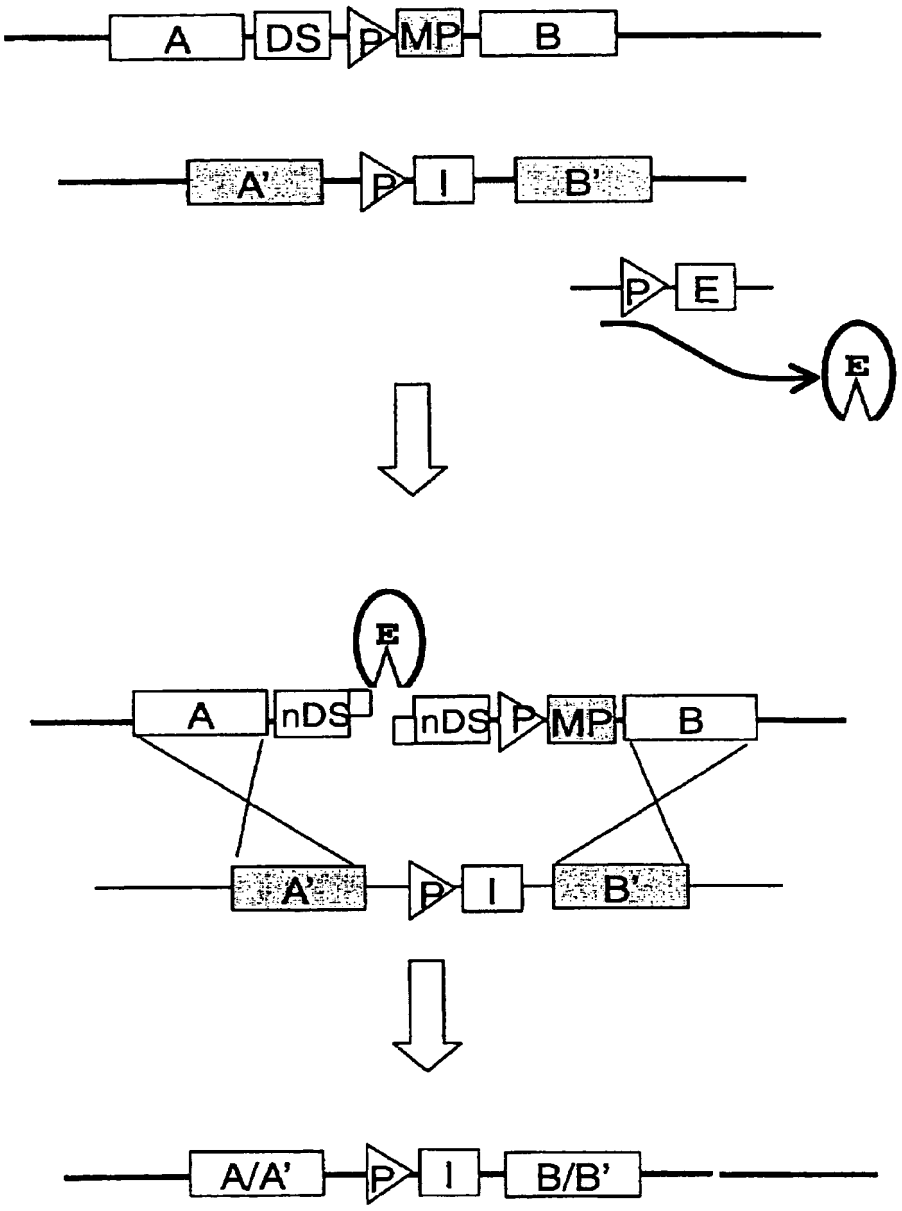

Fig. 5

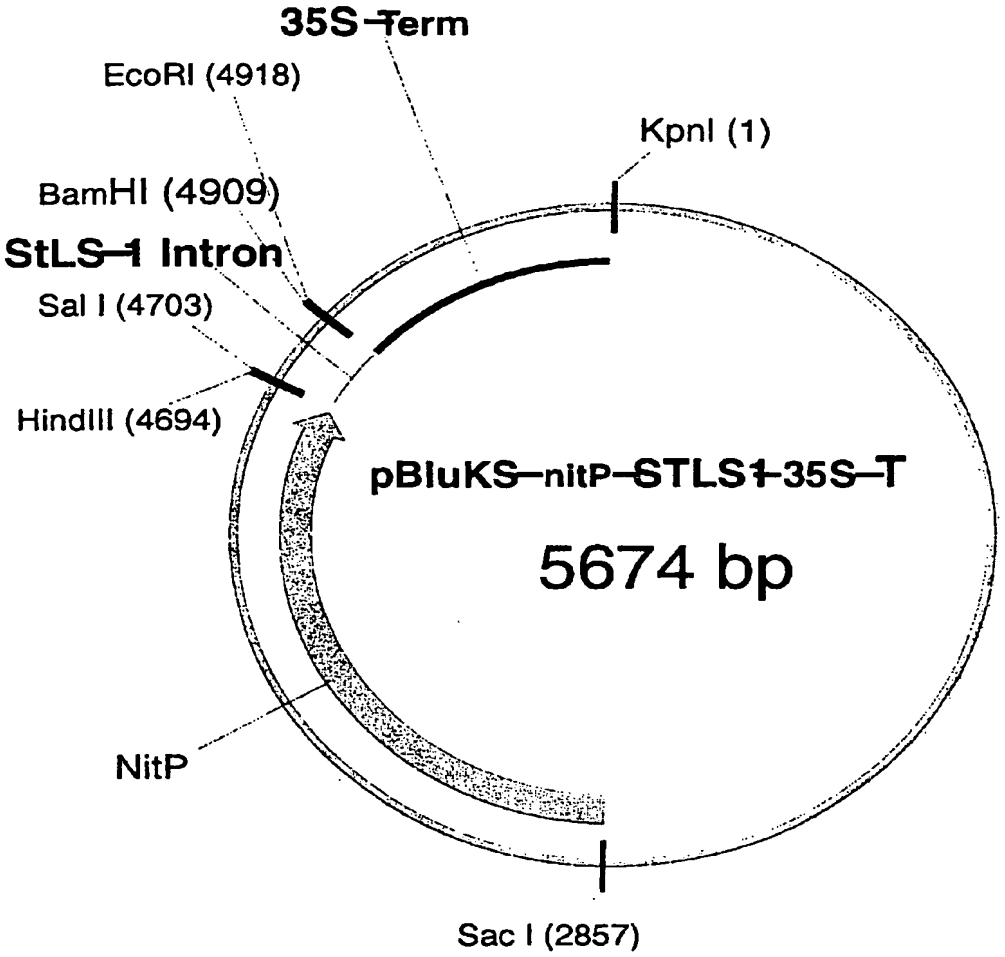
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Fig. 6

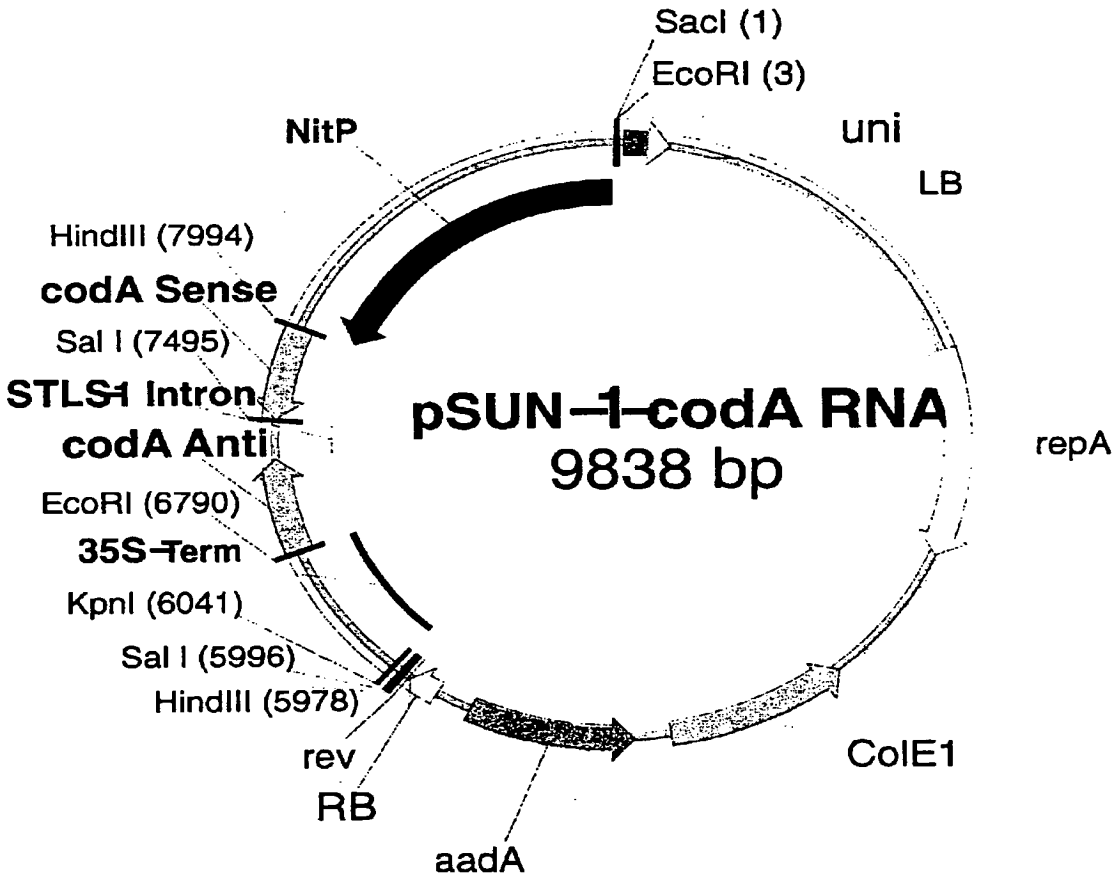
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Fig. 7

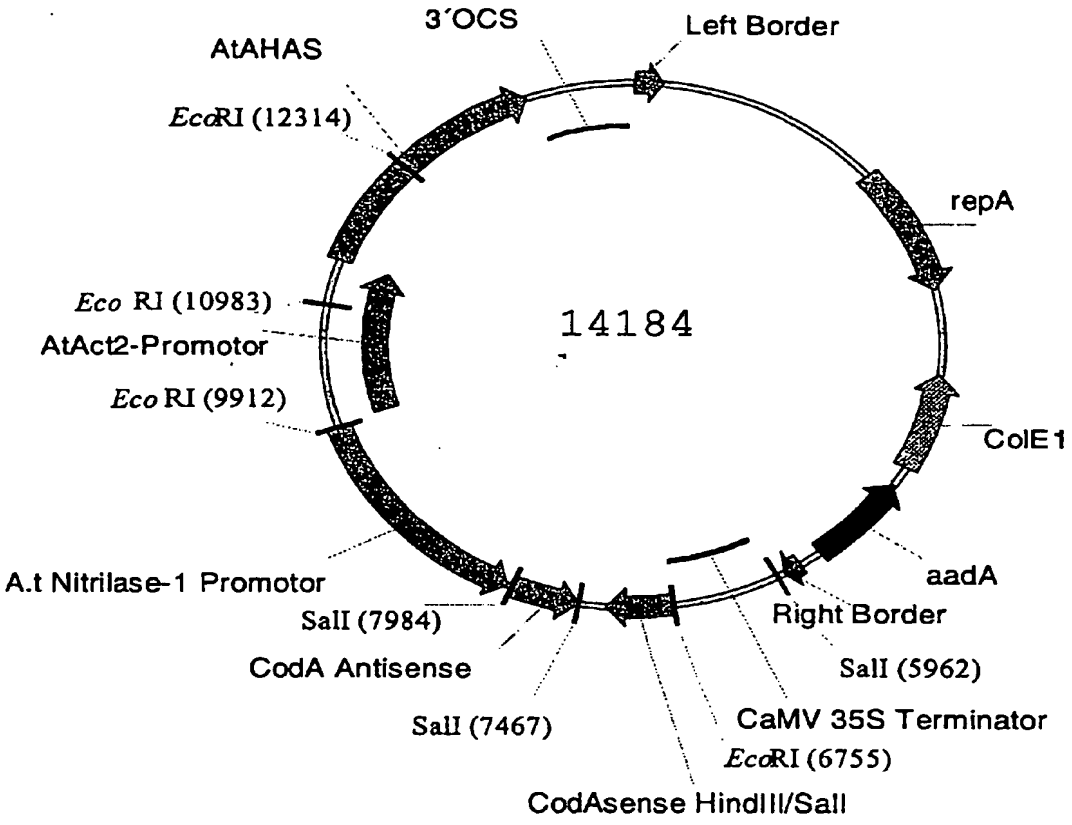

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Fig. 8

```
                    1                                                  50
Klebsiella pneumoniae (1) ---------------------------------MSQYHTFTAHDAVAYAQQ
Clostridium tetani.   (1) -----------------------------MSRFDSHFRMETEDAILYAKE
Zea mays              (1) ARALLSSPLAGASPDCQSASAMAAEEEQGFRPLDESSLLAYIKATPALAS
A.thaliana            (1) ------------------------MSFEEFTPLNEKSLVDYIKSTPALSS
Brassica napus-2      (1) --------------VDDFVLRAKEMSFDEFKPLNEKSLVEYIKATPALSS
Soy-1                 (1) --------------------------------------------------
Oryza sativa-1        (1) --------------------------------------------------
Consensus             (1)                                    L     V    A

                     51                                                 100
Klebsiella pneumoniae (19) FAGIDNPSELVSAQEVGDGNLNLVFKVFDRQGVSRAIVKQALPYVRCVGE
Clostridium tetani.   (22) KLGIFDEHAKLQAEEIGDGNINYVFKVWDVNTKKSVIIKHADIFLRSSGR
Zea mays              (51) RLGGGGSLDSIEIKEVGDGNLNFVYIVQSEAGA--IVVKQALPYVRCVGD
A.thaliana            (27) KIGADKSDDDLVIKEVGDGNLNFVFIVVGSSGS--LVIKQALPYIRCIGE
Brassica napus -2     (37) RLGDKY--DDLVIKEVGDGNLNFVFIVVGSTGS--LVIKQALPYIRCIGE
Soy -1                 (1) --------------------------------------------------
Oryza sativa -1        (1) --------------------------------------------------
Consensus             (51) KLG     D L   EVGDGNLNFVF V    G   LVIKQALPYIRCIGE

                     101                                                150
Klebsiella pneumoniae  (69) SWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMVMEDLS-DHR
Clostridium tetani.    (72) --ELDVDRNRIEAEVLMLQGILAPGLVPKVYKYDSVMCNLSMEDIS-DHR
Zea mays               (99) SWPMTRERAYFEASTLREHGRLCPEHTPEVYHFDRTLSLMGMRYIEPPHI
A.thaliana             (75) SWPMTKERAYFEATTLRKHGNLSPDHVPEVYHFDRTMALIGMRYLEPPHI
Brassica napus -2      (83) SWPMTKERAYFEATTLRKHGGLSPDHVPEVYHFDRTMALIGMRYLEPPHI
Soy -1                  (1) ----------------------IPEHVPEVYHFDRTMSLIGMRYLEPPHI
sativa -1               (1) --------------------------------------------------
Consensus             (101) SWPMT ERA  EA TL  HG LSPDHVPEVYHFDRTMALIGMRYLEPPHI

                     151                                                200
Klebsiella pneumoniae (118) IWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFIN-
Clostridium tetani.   (119) NLRKELLKRNTFPSFAEHITTFIVDTLLPTTDLVMDSGEKKDNVKKYIN-
Zea mays              (149) ILRKGLVAGVEYPLLADHMSDYMAKTLFFTSLLYNNTTDHKNGVAKYSAN
A.thaliana            (125) ILRKGLIAGIEYPFLADHMSDYMAKTLFFTSLLYHDTTEHRRAVTEFCGN
Brassica napus -2     (133) ILRKG-------------------------------------------
Soy -1                 (29) ILIKGLIAGIEYPFLAEHMADFMAKTLFFTSLLFRSTADHKRDVAEFCGN
Oryza sativa -1         (1) --------------------------------LLYNSTTDHKKGVAQYCDN
Consensus             (151) ILRKGLIA I YP  ADHM DYMA TLF TSLLY  T DHK  VA F  N

                     201                                                250
Klebsiella pneumoniae (167) PAMCEITEDLFFNDPYQIHERN--NYPAELEADVAALRDDAQLKLAVAAL
Clostridium tetani.   (168) KDLCKISEDLVFTEPFIDYKSRNTVLEENIEFVKRQLYEDKELILEAGKL
Zea mays              (199) VEMCRLTEQVVFSDPYRVSKFNR-WTSPYLDKDAEAVREDDELKLEVAGL
A.thaliana            (175) VELCRLTEQVVFSDPYRVSTFNR-WTSPYLDDDAKAVREDSALKLEIAEL
Brassica napus -2     (138) -------------------------------------------------
Soy -1                 (79) VELCRLTEQVVFSDPYKVSQYNR-WTSPYLDRDAEAVREDNLLKLEVAEL
Oryza sativa -1        (20) VEMCRLTEQVVFSDPYMLAKYNR-CTSPFLDNDAAAVREDAELKLEIAEL
Consensus             (201) VELCRLTEQVVFSDPY VS FNR  TSPYLD DA AVRED  LKLEVA L
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Fig. 9a

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 251                                                  300
Klebsiella pneumoniae      (215) KHRFFAHAEALLHGDIHSGSIFVAEGSLKAIDAEFGYFGPIGFDIGTAIG
Clostridium tetani.        (218) KNNFMNNSQALIHGDLHSGSIFVNEESTKILDPEFAFYGPIGYDLGNVIG
Zea mays                   (248) KSMFIERAQALIHGDLHTGSIMVTEVQLKSLIQNLGSMGPMGFDIGSLPW
A.thaliana                 (224) KSMFCERAQALIHGDLHTGSVMVTQDSTQVIDPEFSFYGPMGFDIGAYLG
Brassica napus -2          (138) --------------------------------------------------
Soy -1                     (128) KSKFIES-----------------------------------------
Oryza sativa -1             (69) KSMFIERAQALLHGDLHTGSIMVTPDSTQVIDPEFAFYGPMGYDIGAFLG
Consensus                  (251) KS FIE AQALIHGDLHTGSI V   S   ID EFAFYGPMGFDIG  IG

                            301                                                  350
Klebsiella pneumoniae      (265) NLLLNYCGLPGQLGIRDAAAAREQRLNDIHQLWTTFAERFQALAAEKTRD
Clostridium tetani.        (268) NLFFAWANAYVTEDGKEVEEFTIWIEKTIENILELFKEKFIKKYKEIVTD
Zea mays                   (298) KPDFGHTMHRMGMLIKRMIVRLTRMDLEDN------------------
A.thaliana                 (274) NLILAFFAQDGHATQENDRKEYKQWILRTIEQTWNLFNKRFIALWDQNKD
Brassica napus -2          (138) --------------------------------------------------
Soy -1                     (135) --------------------------------------------------
Oryza sativa -1            (119) NLILAYFSQDGHADQANDRKAY--------------------------
Consensus                  (301) NL  AY

                            351                                                  400
Klebsiella pneumoniae      (315) AALAYPGYASAFLKKVWADAVGFCGSELIRRSVGLSHVADIDTIQDDAMR
Clostridium tetani.        (318) VMAKEEYYMNWYLHSILSDTAGQVGLEIIRRVVGDSKVLDITSITDINKR
Zea mays                   (328) --------------------------------------------------
A.thaliana                 (324) GPGEAYLADIYNNTEVLKFVQENYMRNLLHDSLGFGAAKMIRRIVGVAHV
Brassica napus -2          (138) --------------------------------------------------
Soy -1                     (135) --------------------------------------------------
Oryza sativa -1            (141) --------------------------------------------------
Consensus                  (351)

                            401                                                  447
Klebsiella pneumoniae      (365) HECLRHAITLGRALIVLAERIDSVDELLARVRQYS------------
Clostridium tetani.        (368) VKAERILILSAKTFIKNRHKIKTGKRYVEIFNSNMY------------
Zea mays                   (328) --------------------------------------------------
A.thaliana                 (374) EDFESIEEDKRRAICERSALEFAKMLLKERRKFKSIGEVVSAIQQQS
Brassica napus -2          (138) --------------------------------------------------
Soy -1                     (135) --------------------------------------------------
Oryza sativa -1            (141) --------------------------------------------------
Consensus                  (401)
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Fig. 9b

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